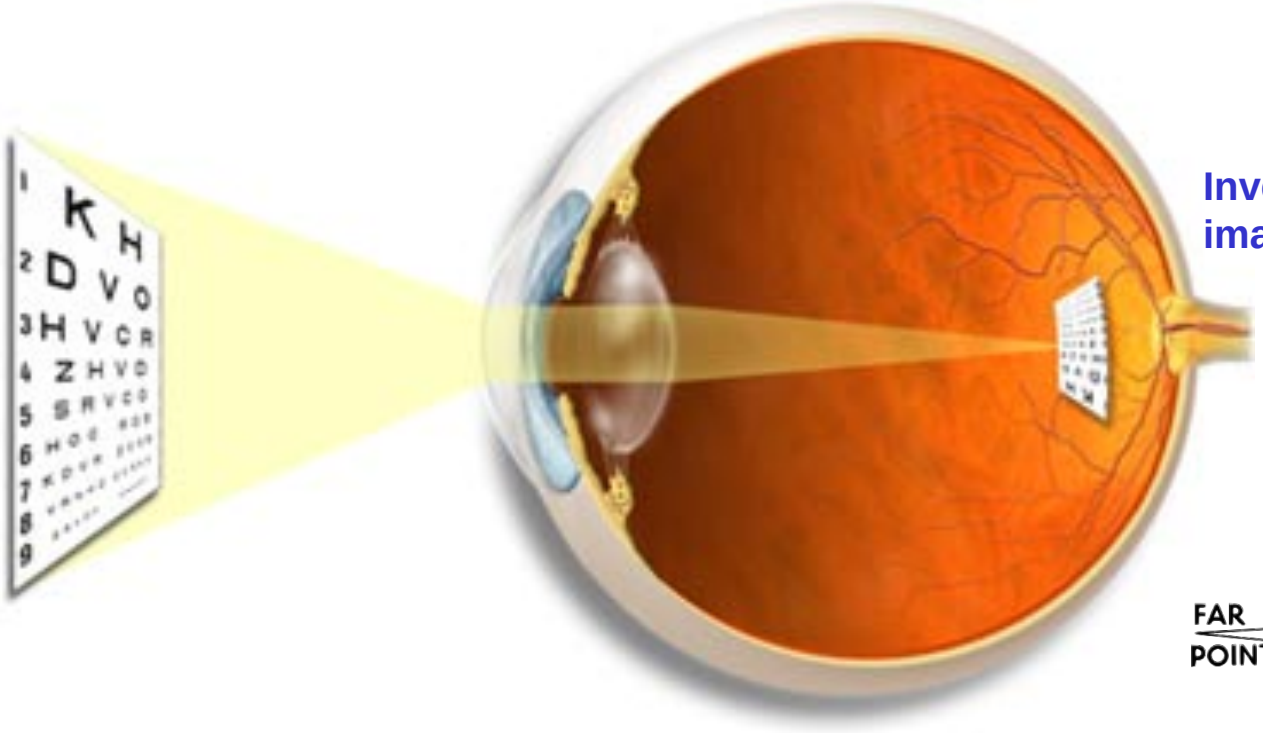
Retina - Introduction

Dr. Anthony Vugler

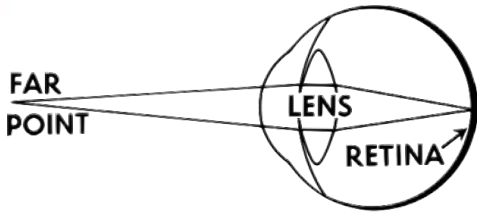
UCL-Institute of Ophthalmology

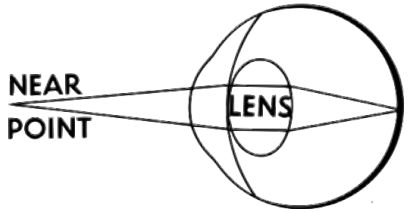
Introduction to the retina

An image of an object is focused by the cornea and lens onto the rear surface of the eye: the retina.



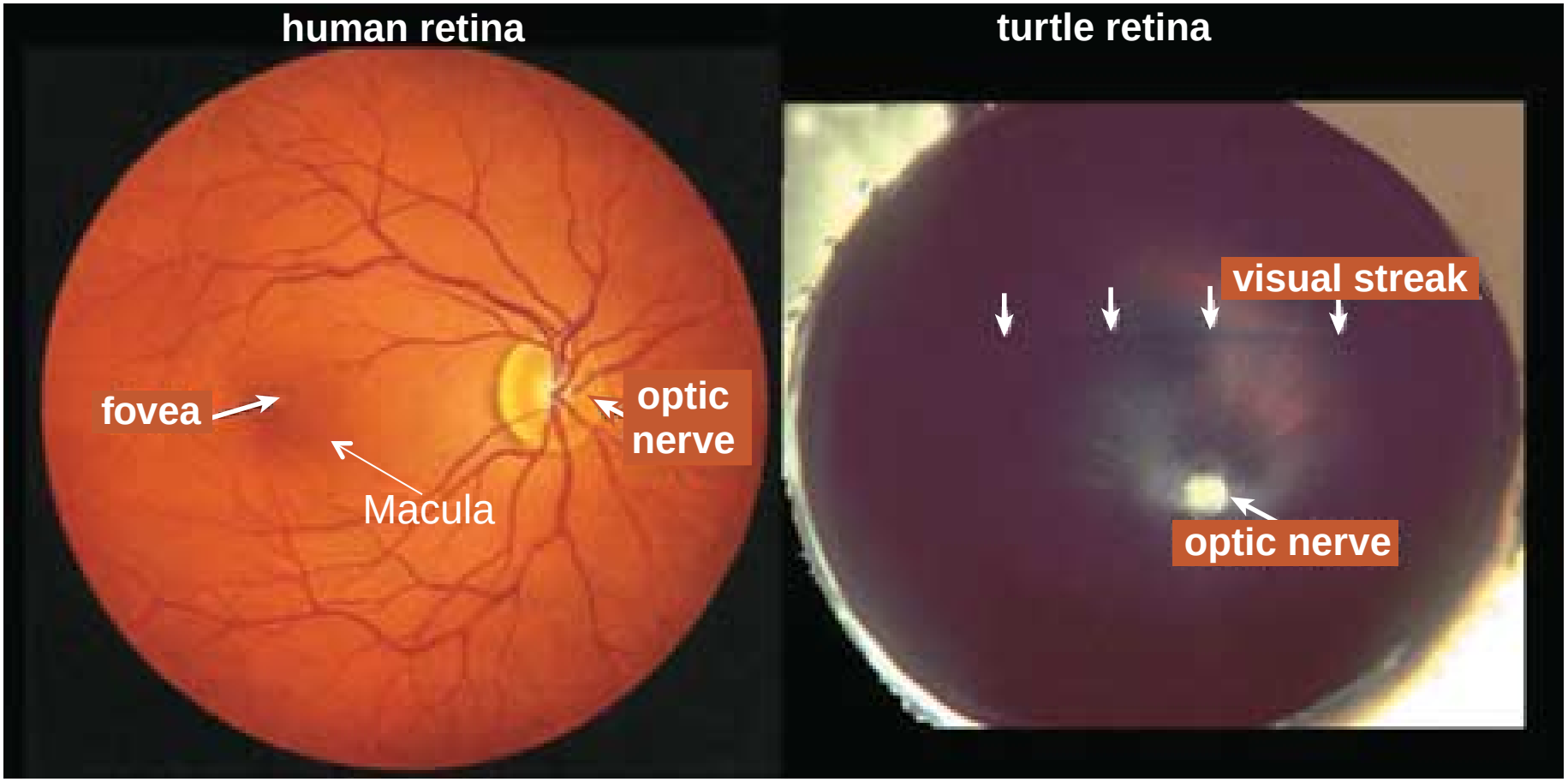
Inverted image





Accommodation: Focus on near objects by contracting Ciliary muscle and changing shape of lens.

Retina – The fovea

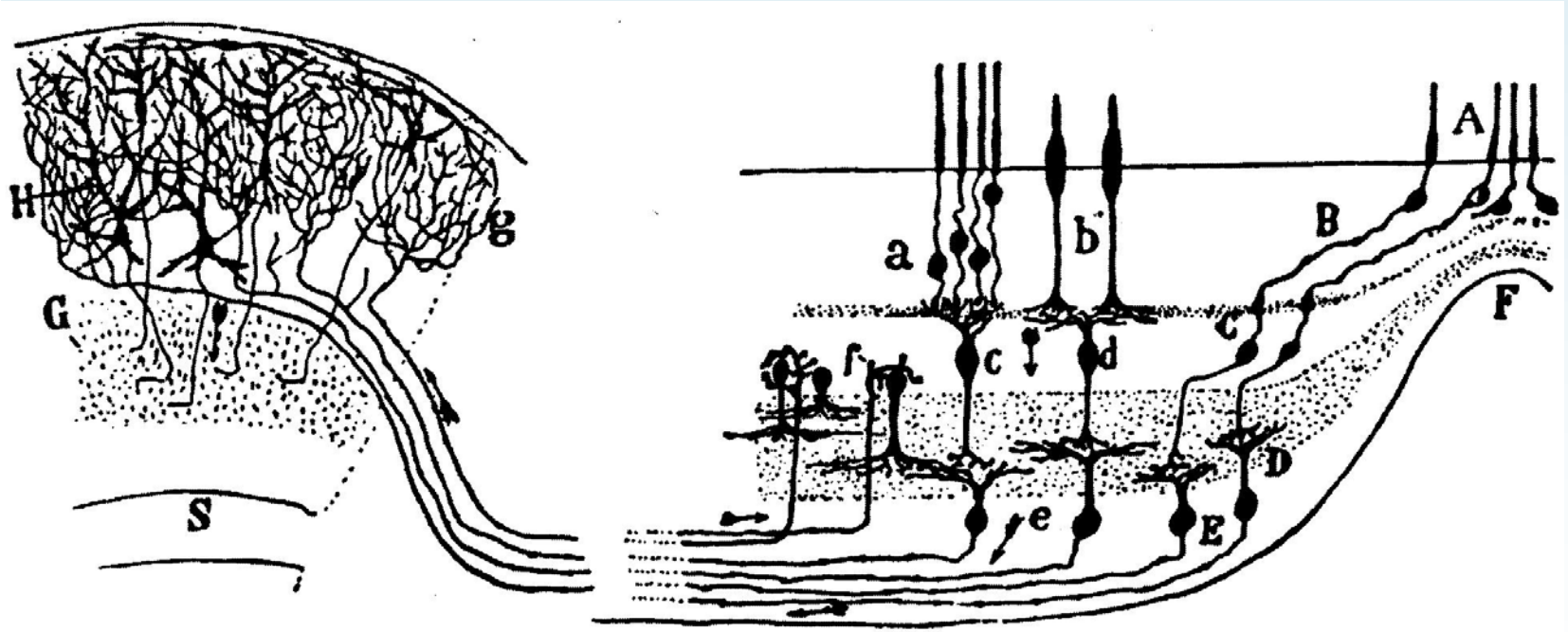


The retina is a transparent plate like structure <0.5mm thick.

The macula lutea is a yellow spot of ~5mm surrounding the fovea with colouration derived from dietary carotenoid (Xanthophyll) pigments.

The retina - Cells

Cajal's (1909-1911) neural architecture drawing based on the Golgi method.



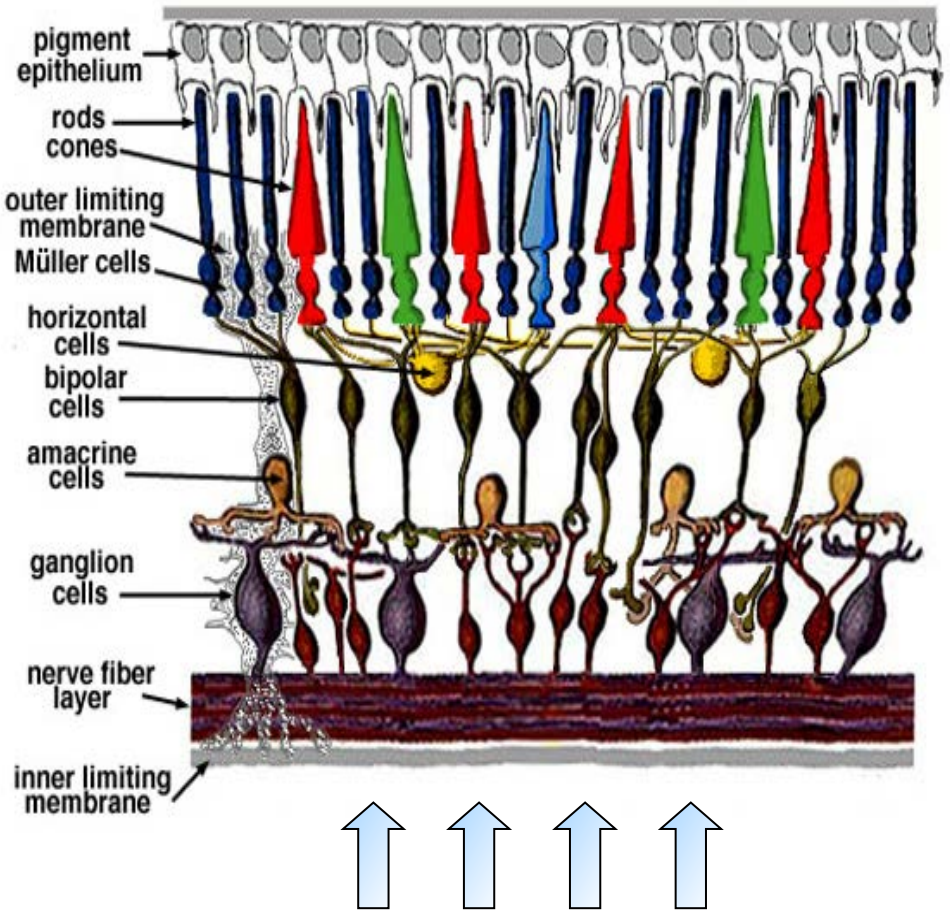
Optic Tectum
(superior colliculus)

Retina

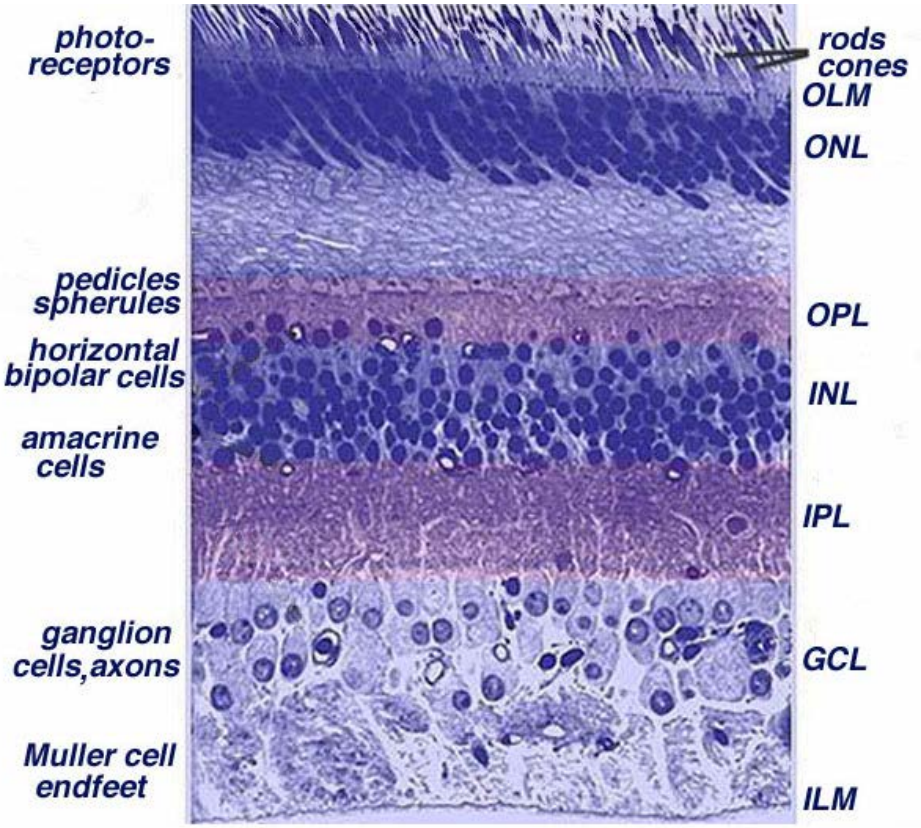
- Ramon y Cajal noted that neurons have anatomical polarity.
- No myelination in the retina
- Myelination of axons in the optic nerve

Retina: A light sensitive part of the CNS

Schematic vertical section



Light Microscopic vertical section

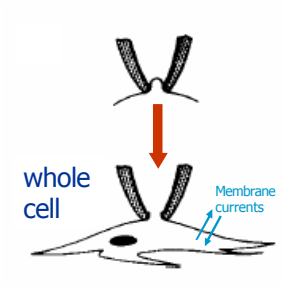


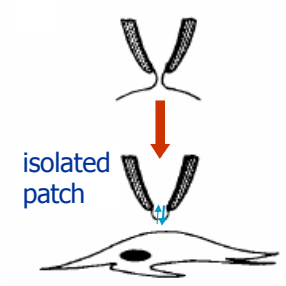
1-4 types horizontal cell
11 types of bipolar cell
22-30 types of amacrine cell
20 types of ganglion cell

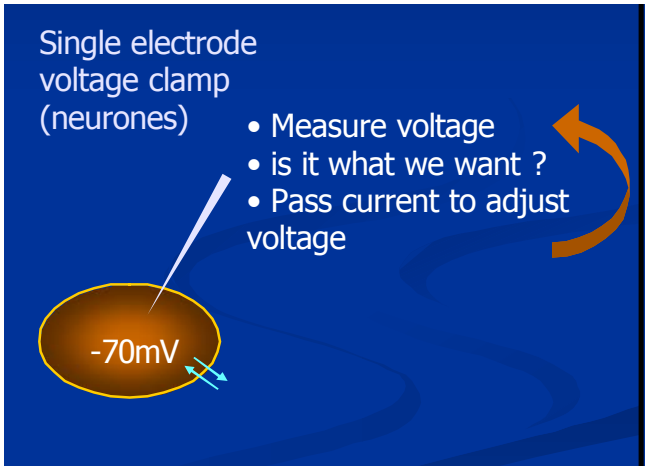
Electrophysiological recording methods

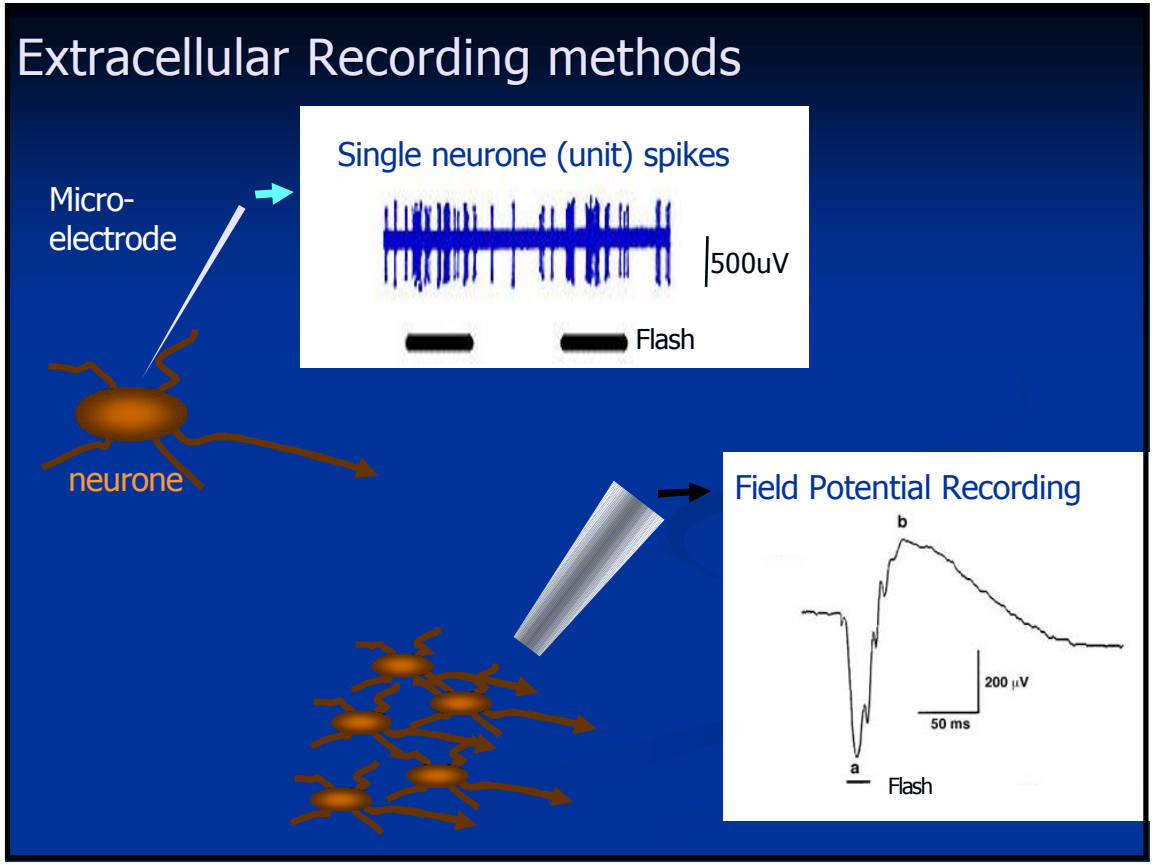
UCL

Whole cell (Patch) recording



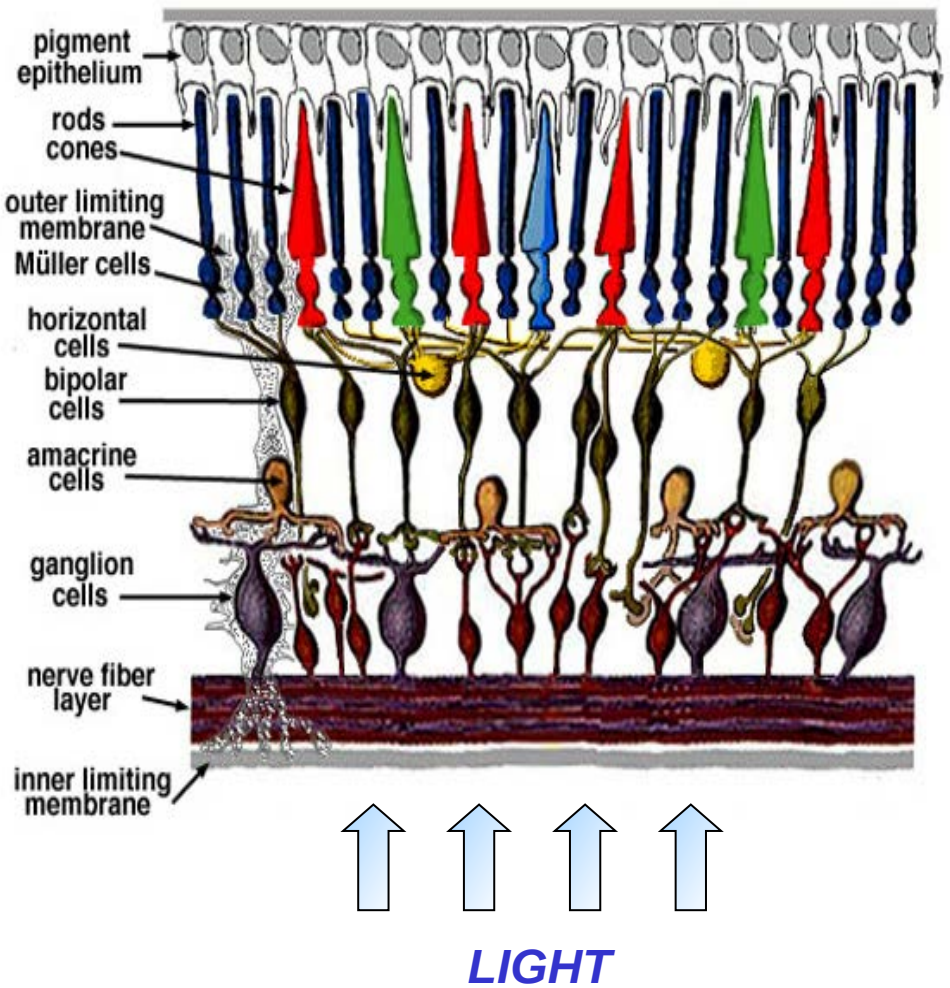




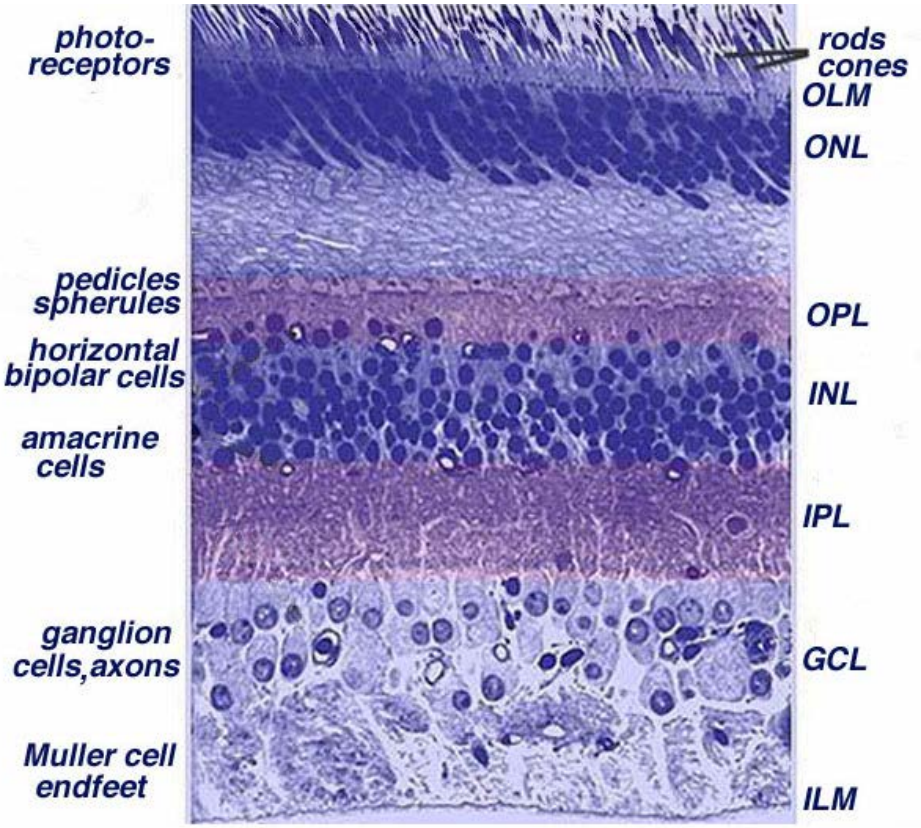


Retinal ganglion cells (output)

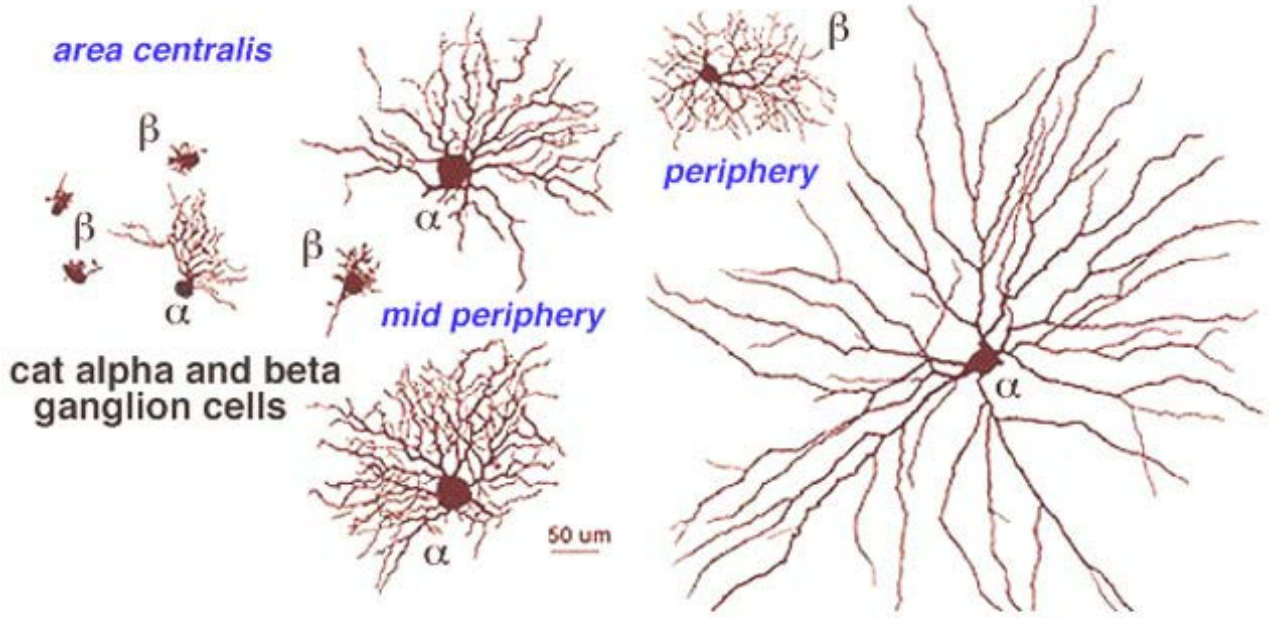
Schematic vertical section



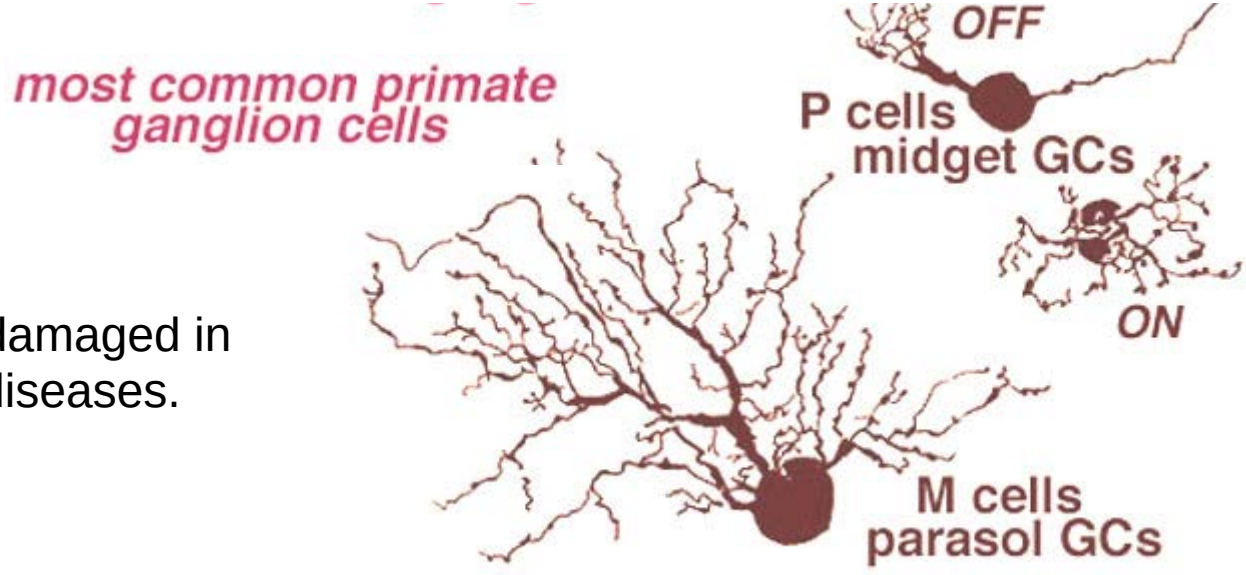
Light Microscopic vertical section



Types of Retinal Ganglion Cells

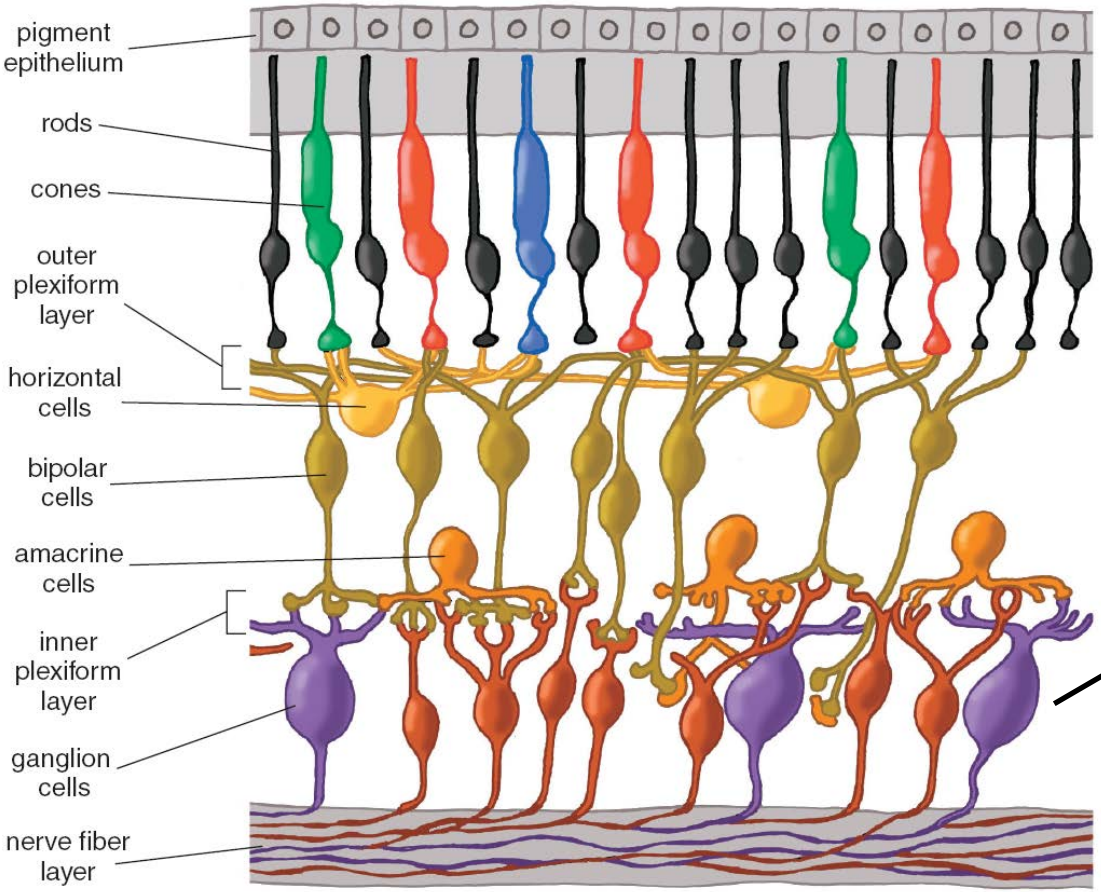


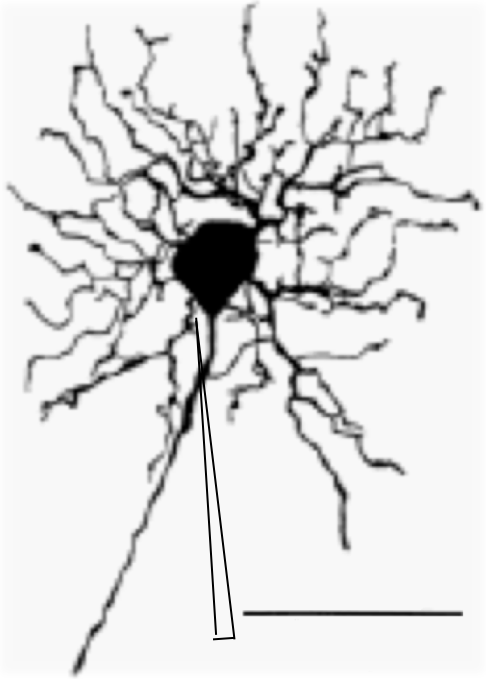
cat alpha and beta ganglion cells



Output cells of the retina, damaged in Glaucoma & other retinal diseases.
~20 different types.

Recording from retinal ganglion cells

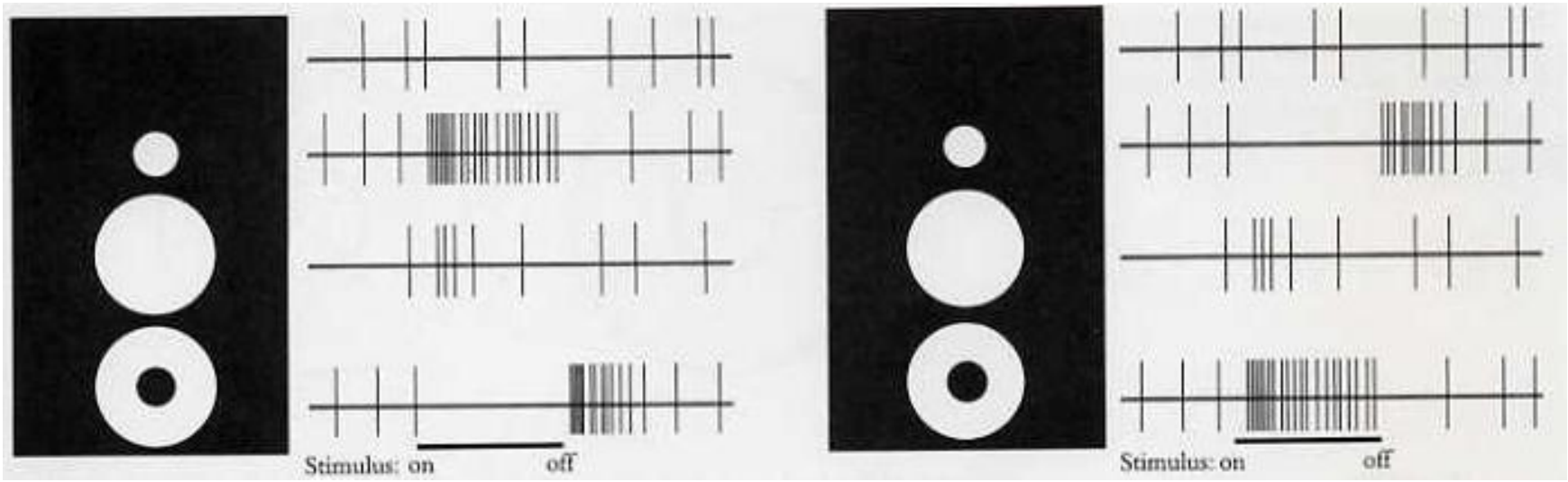




Stephen Kuffler 1950

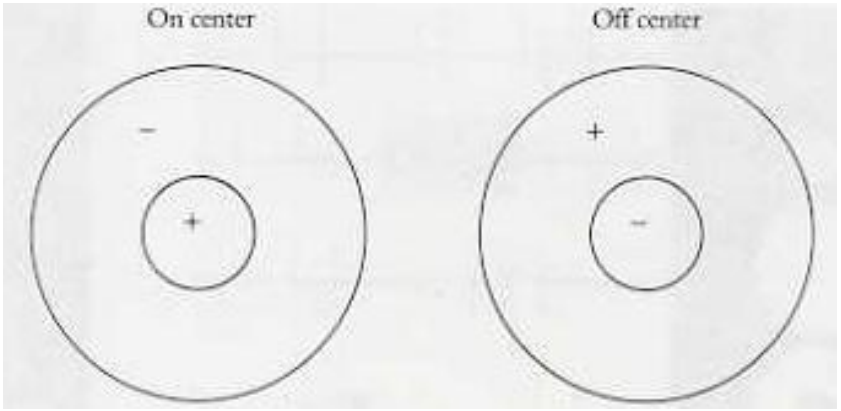
Retinal ganglion cells: output of retina and relatively easy to record from

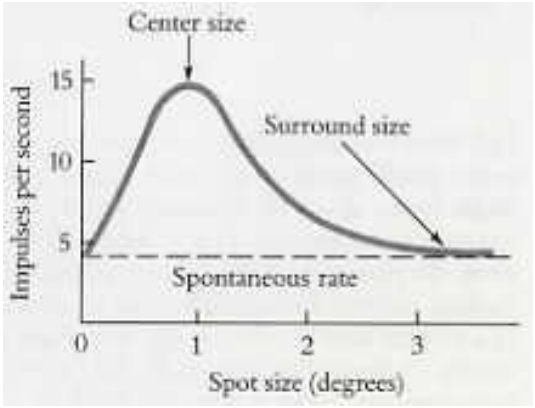
Responses of Retinal Ganglion Cells



ON center

OFF center



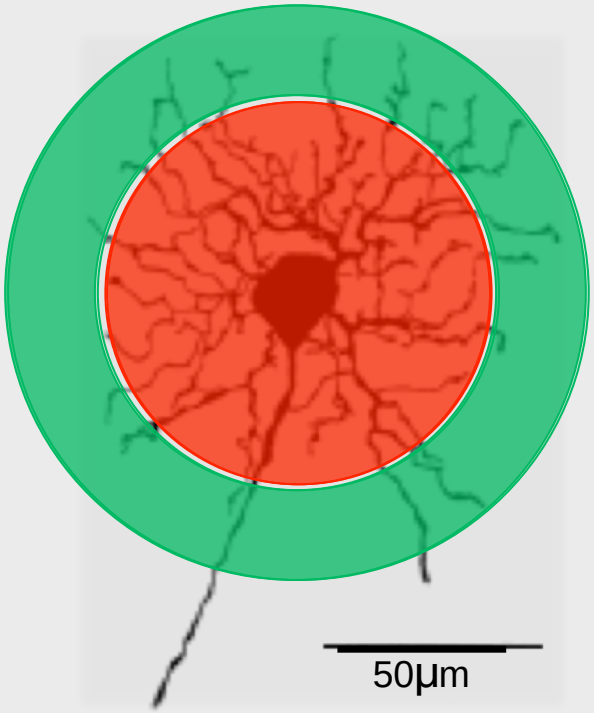


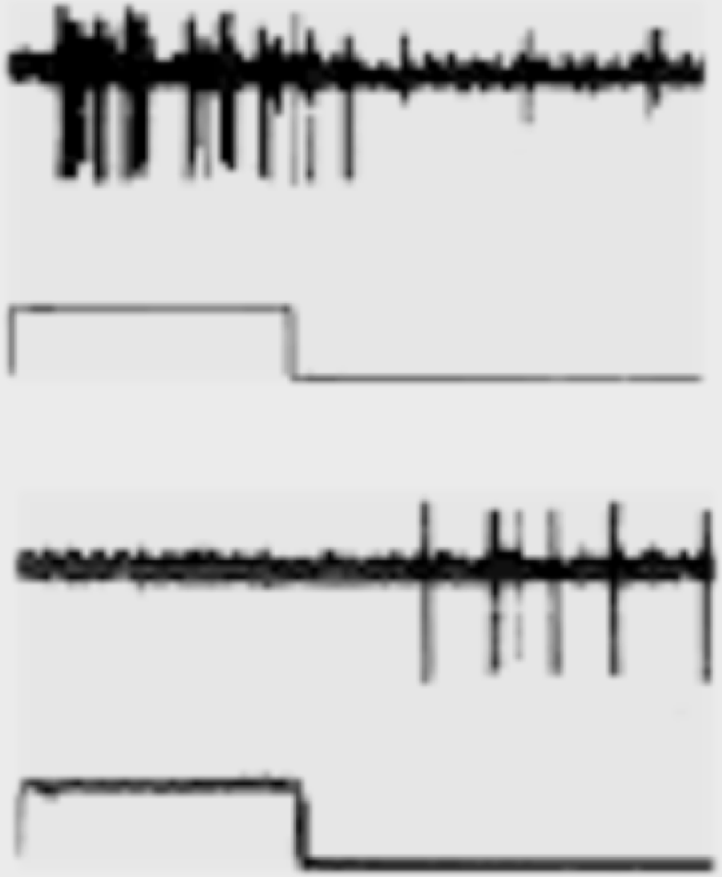
Stephen Kuffler 1950

Retinal Ganglion Cell Receptive Field

ON centre

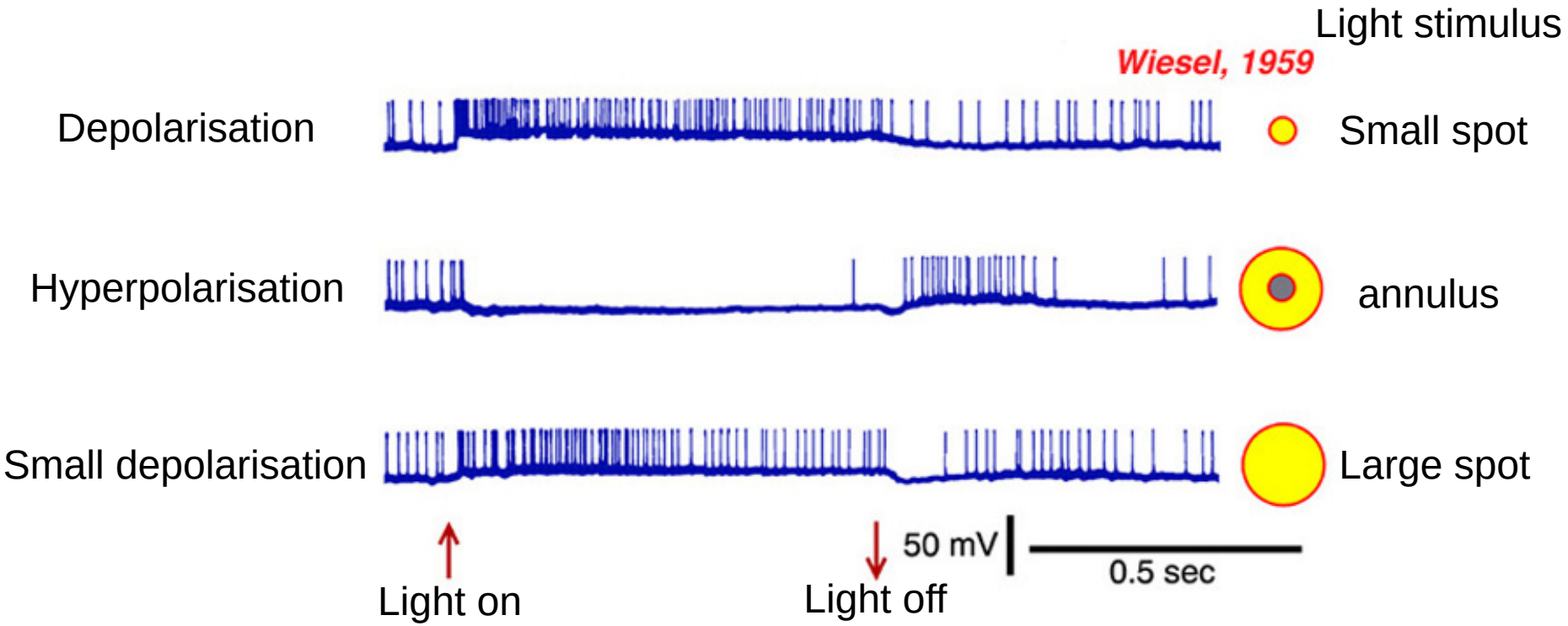
OFF surround





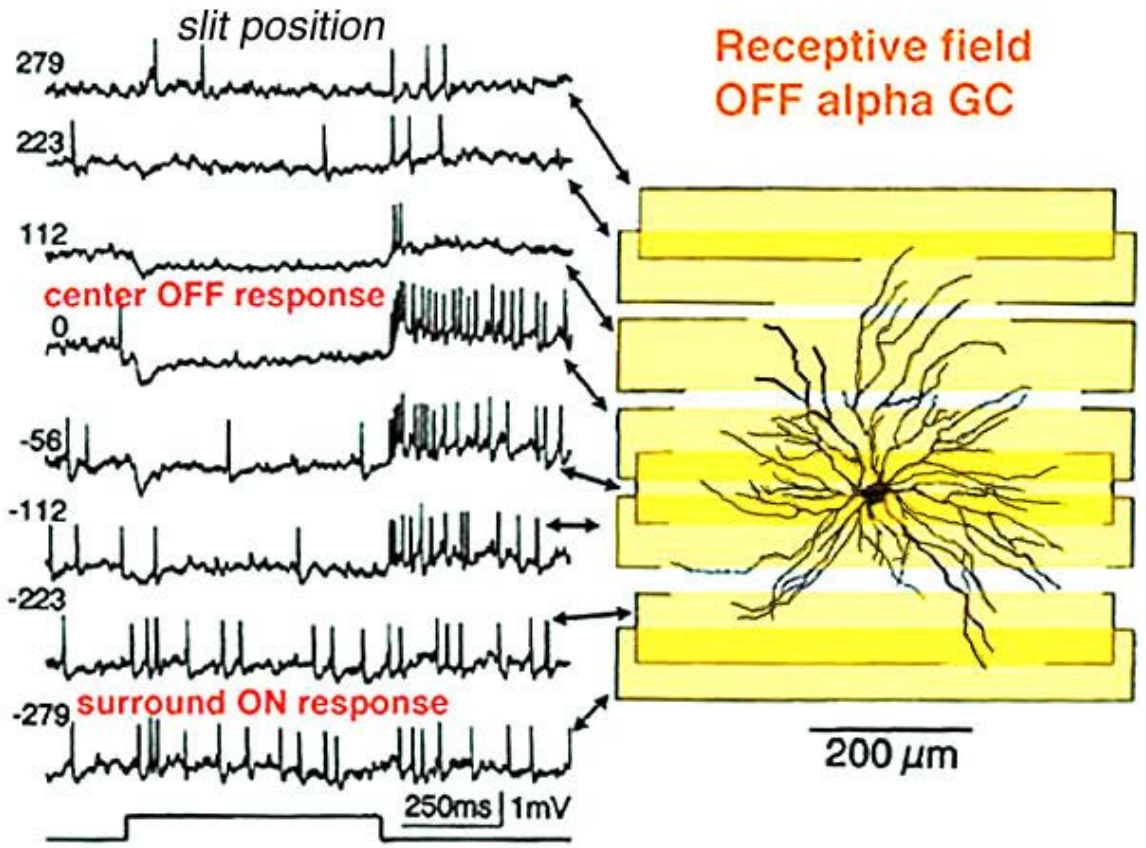
- RGC receptive fields are fixed in space and don't typically exceed 1mm.
- There is graded sensitivity over this region, with the strongest response elicited by a light spot directly over the electrode.

Responses of Retinal Ganglion Cells



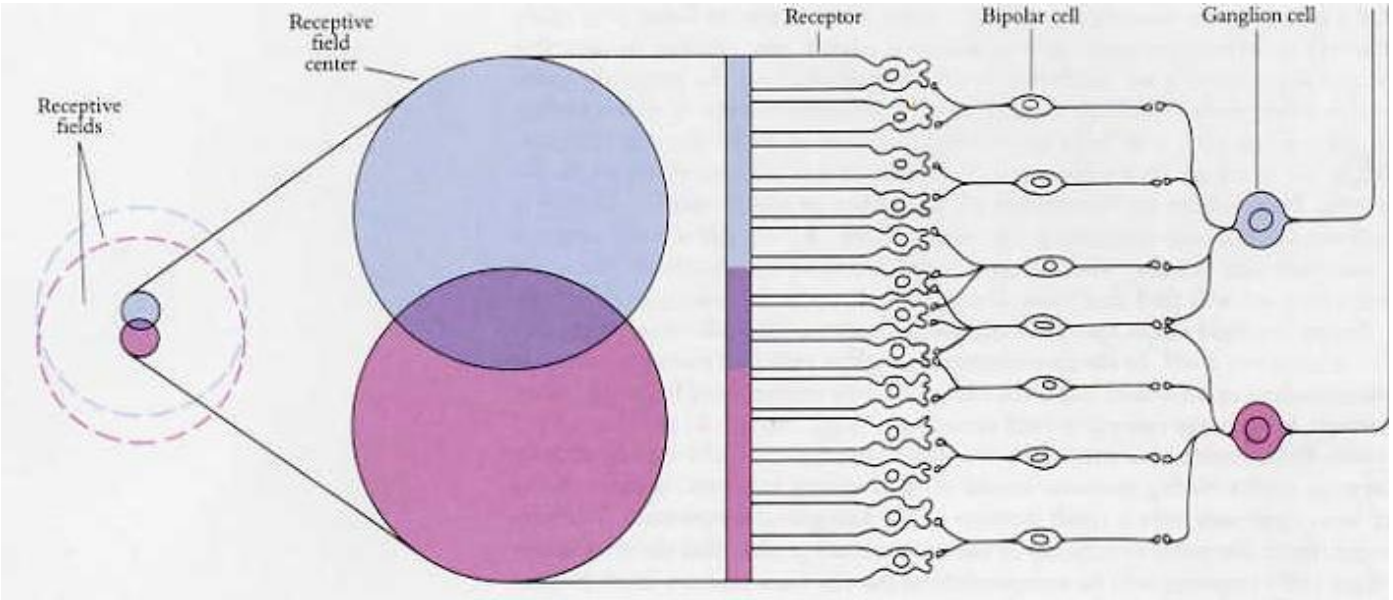
- This center-surround inhibition prevents the lateral spread of activation
- As a consequence, all RGCs respond best to small spots of light

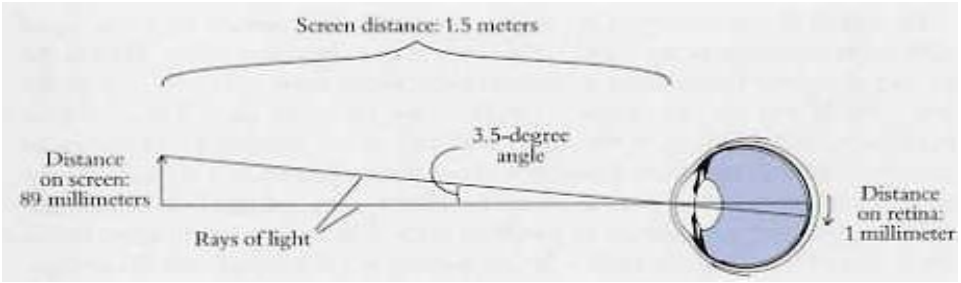
Responses of Retinal Ganglion Cells



Ganglion cell responses can also be elicited by bars of light

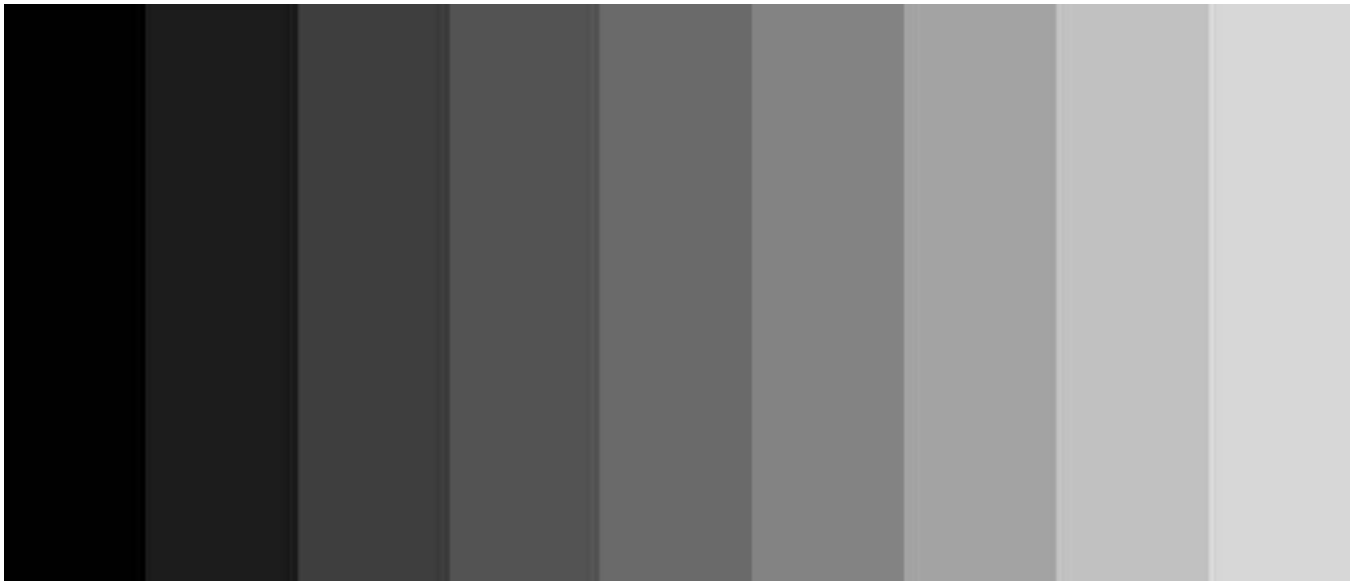
Receptive fields

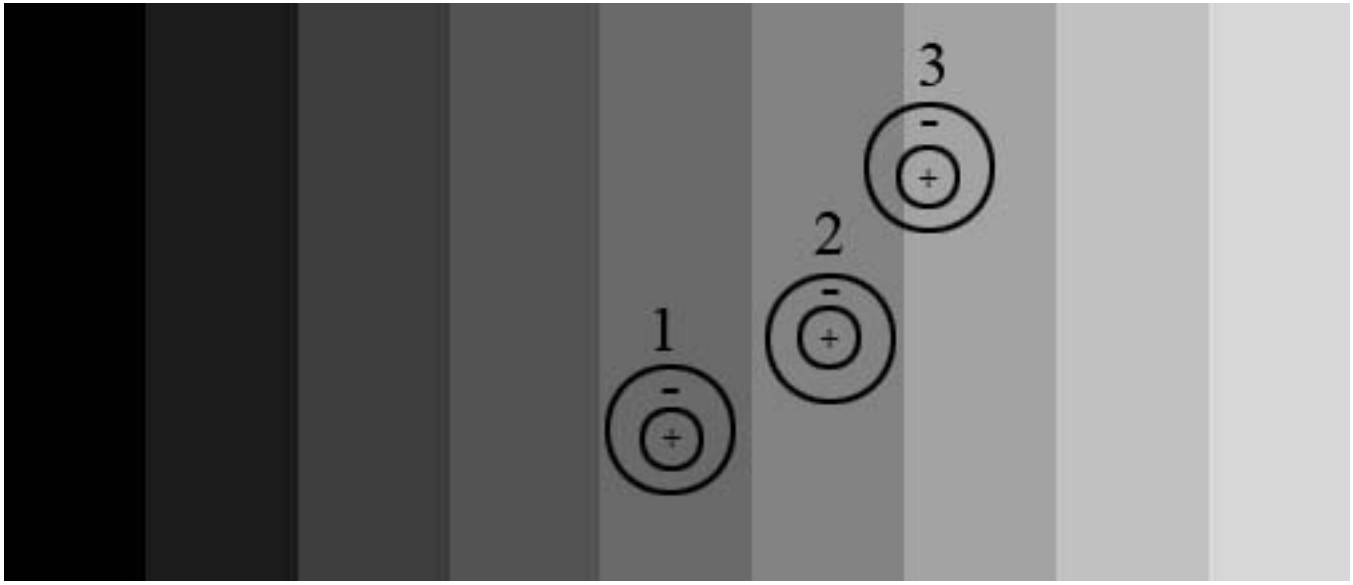




The size of receptive field differs from RGC to RGC. The RGCs in fovea have smallest centers for highest acuity. Larger receptive fields in the retinal periphery result in lower acuity. In monkey The smallest field centers are found in the fovea (0.01mm on retina), generated by handful of cones. In the periphery, receptive fields can be made from 000's of photoreceptors.

Mach Bands





How do signals from photoreceptors produce these characteristic ganglion cell responses?

- Photoreceptors
- Bipolar cells
- Amacrine cells
- Horizontal cells

Photoreceptors: Rod & Cones

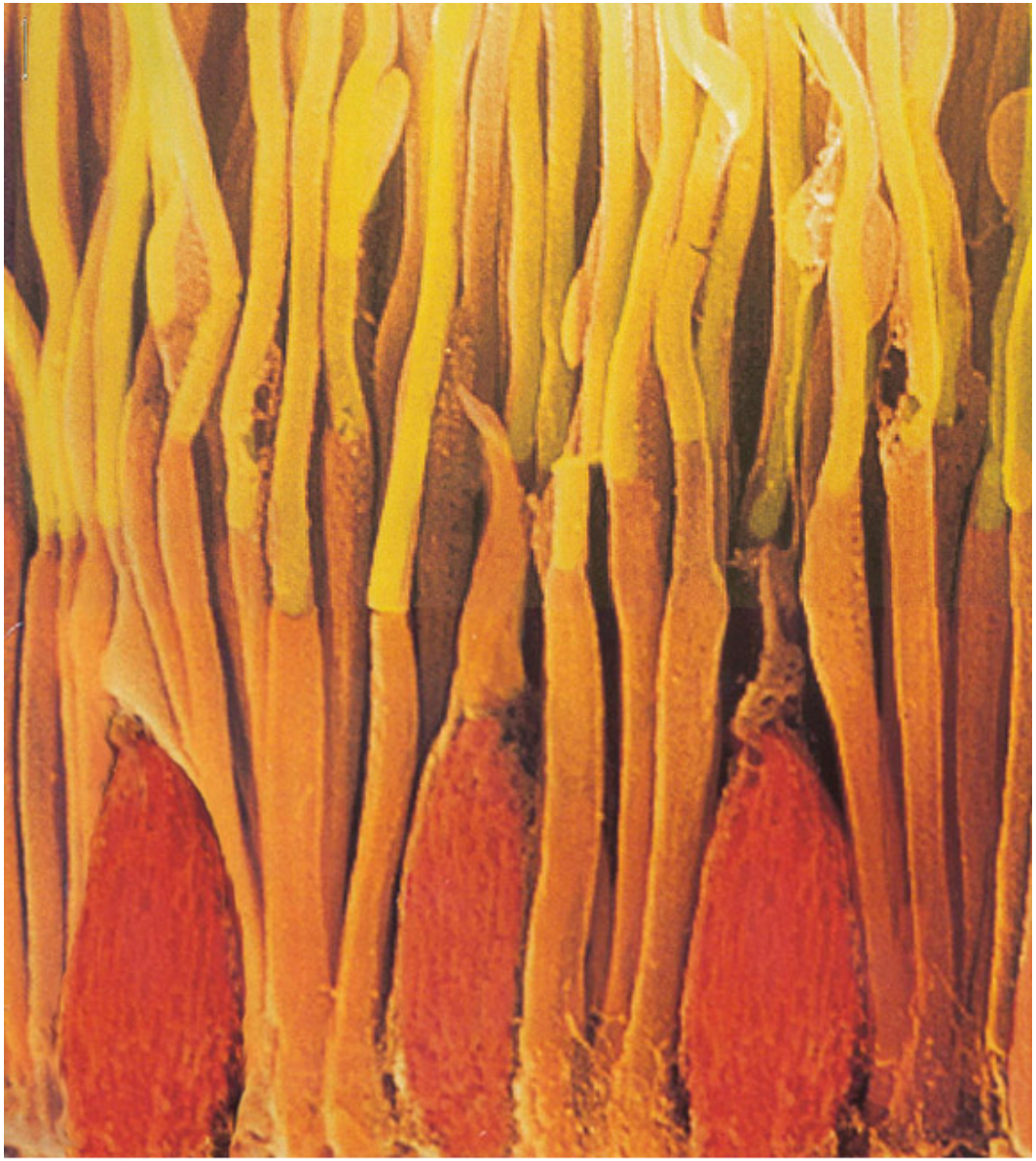
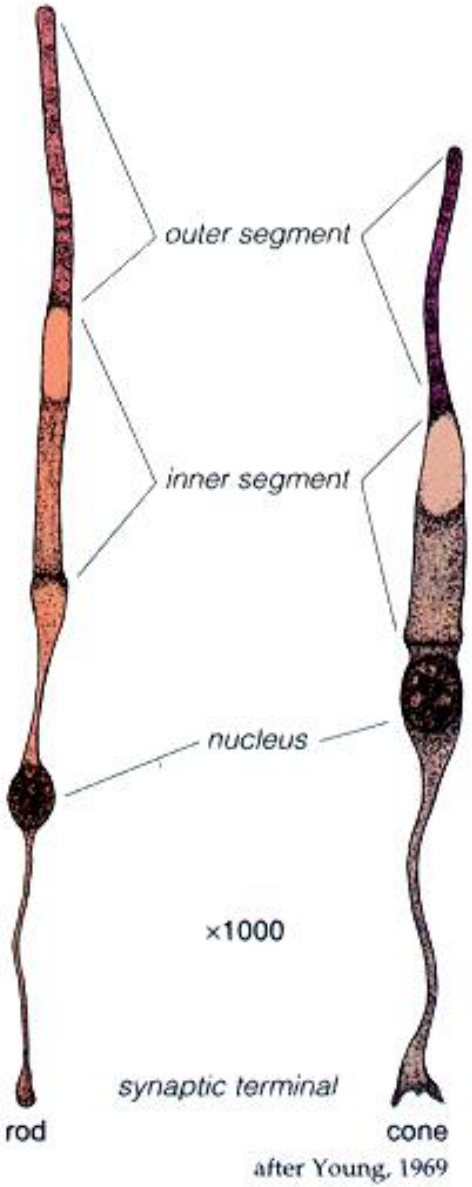
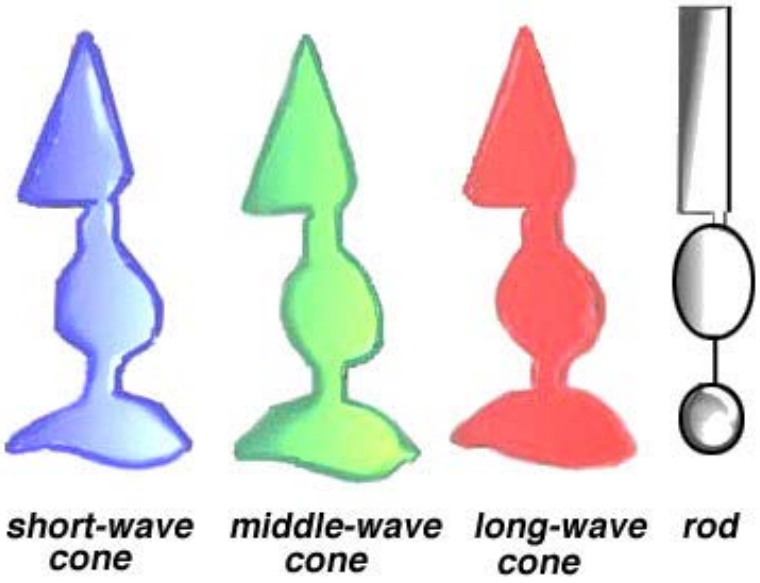


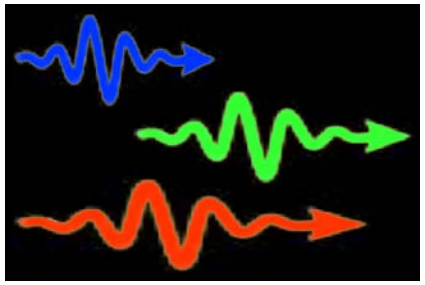
Fig1b. Scanning electron micrograph of the rods and cones of the primate retina. Image adapted from one by Ralph C. Eagle/Photo Researchers, Inc.

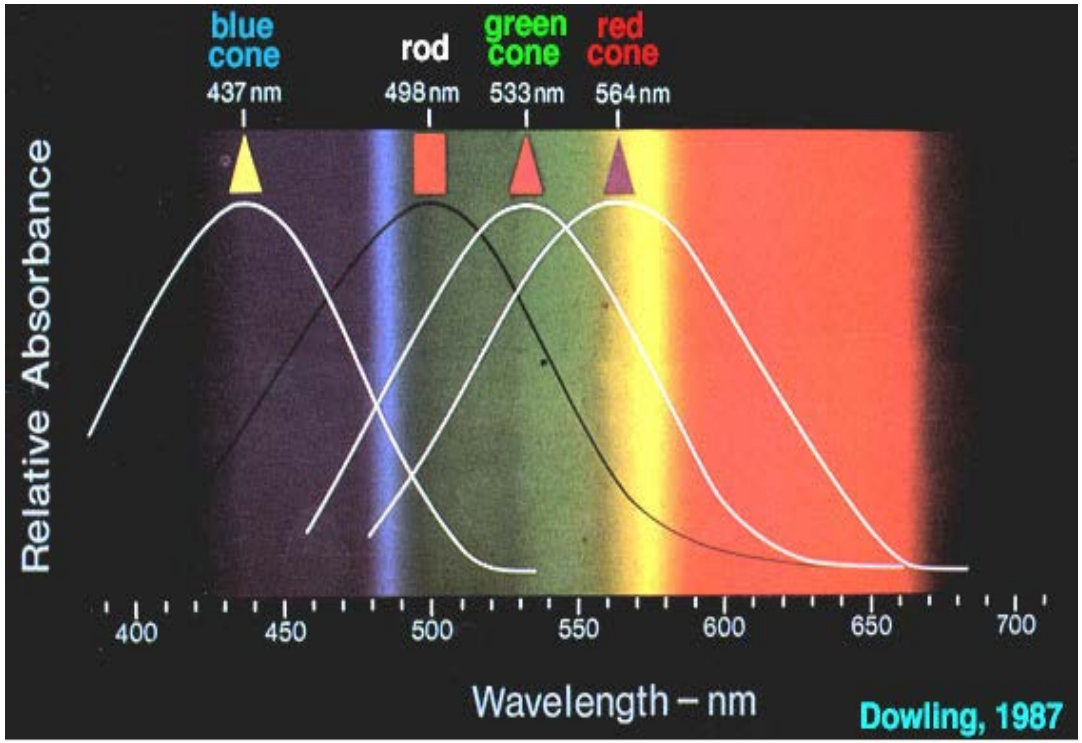


after Young, 1969

Photoreceptors



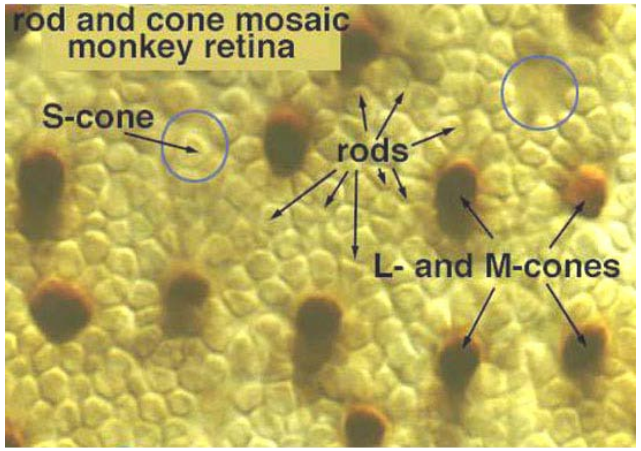


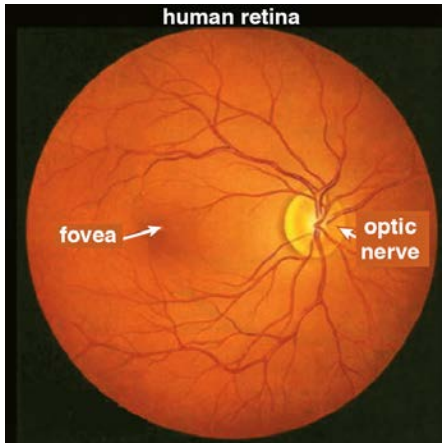


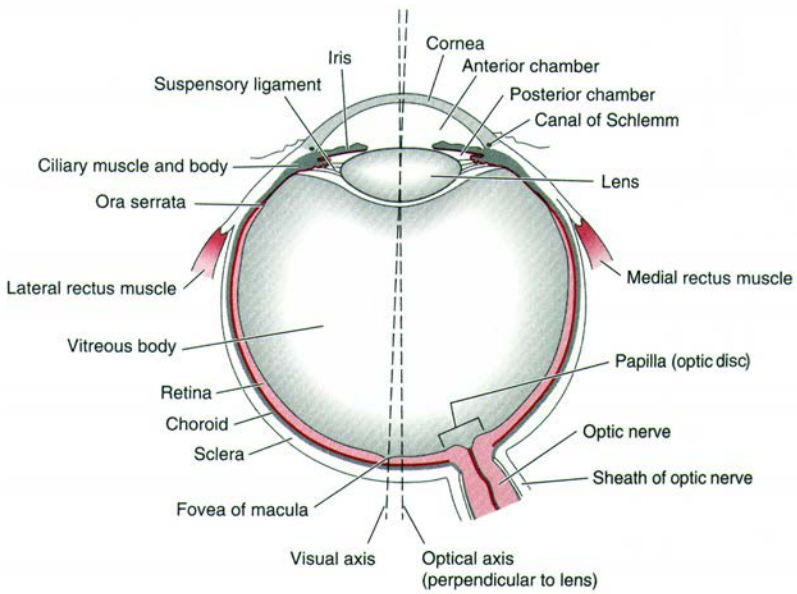
The human retina processes photonic information using four photoreceptor types in the outer nuclear layer (ONL): Short-wavelength (S, blue) cones, middle/medium wavelength cones (M, green), long wavelength cones (L, red) and rods.

Dim / Dark (scotopic) conditions – rod function dominates
Light / ”normal” (photopic) conditions – cone function dominates

Rod / Cone Distribution

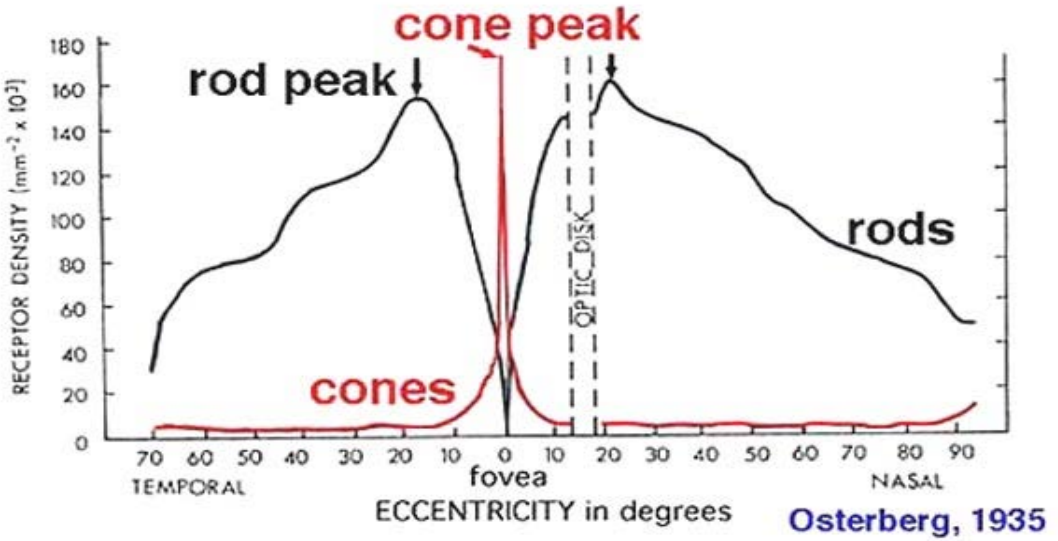






Rods always form a hexagonal packing around the cones and separate the cones from each other

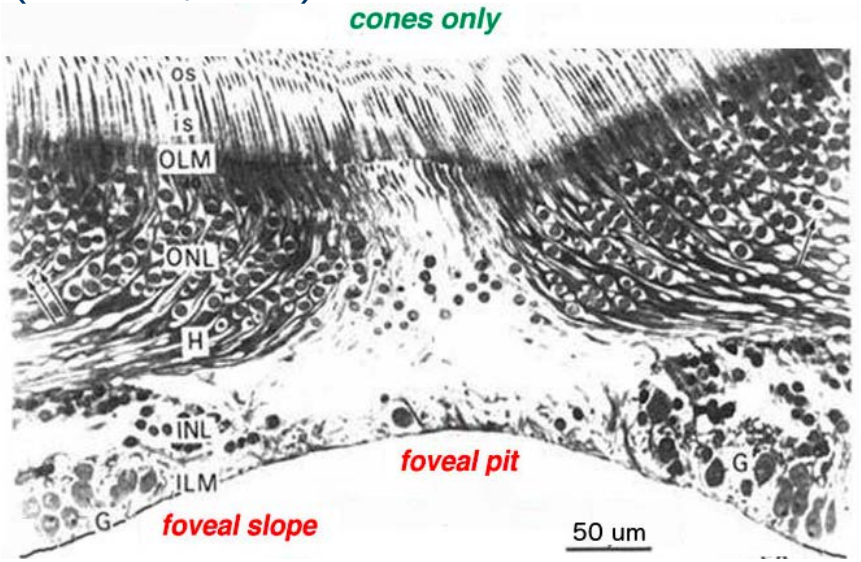
Rods peak in density in a ring approximately 5mm (18 degrees) from the center of the fovea (human/rhesus monkey). Rods are present in greater numbers than cones from 2 mm from the fovea to the far periphery.



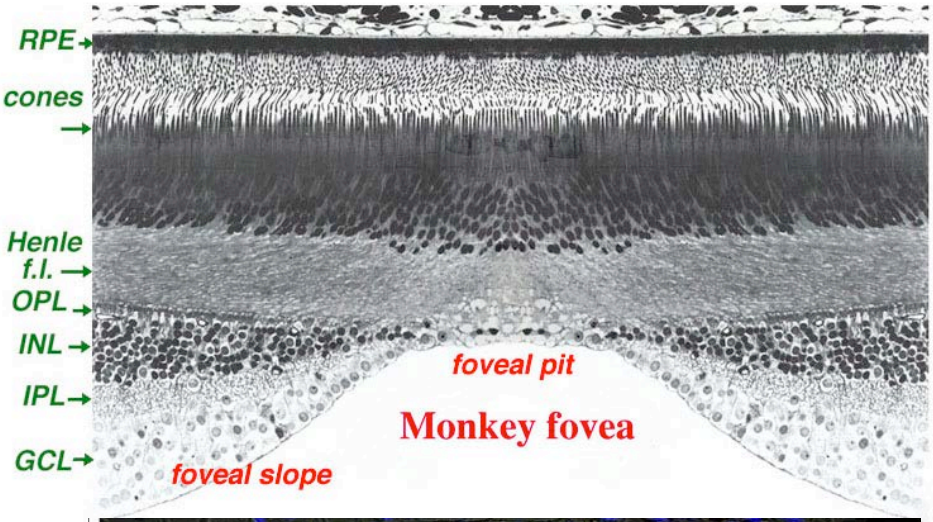
The Fovea

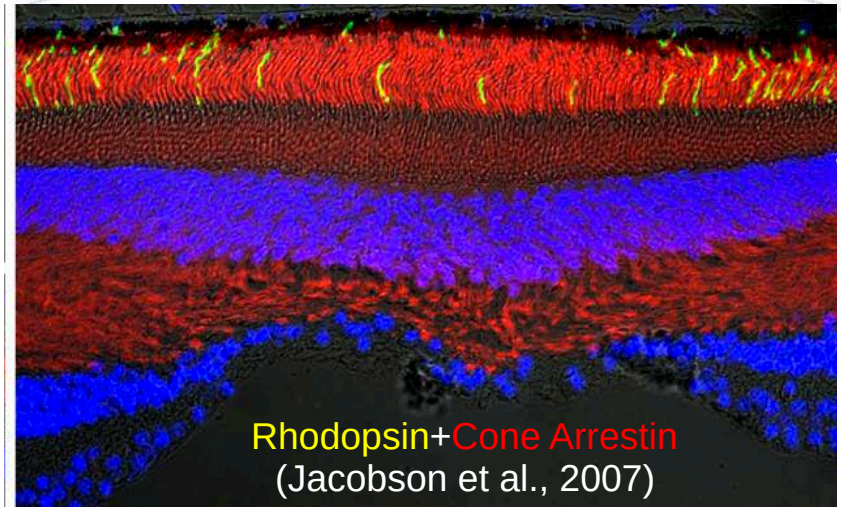
The fovea lies in the middle of the macula area of the retina to the temporal side of the optic nerve head

Vertical section of the human fovea (Yamada, 1969).



Vertical section of the monkey fovea (Hagerman & Johnson, 1991)



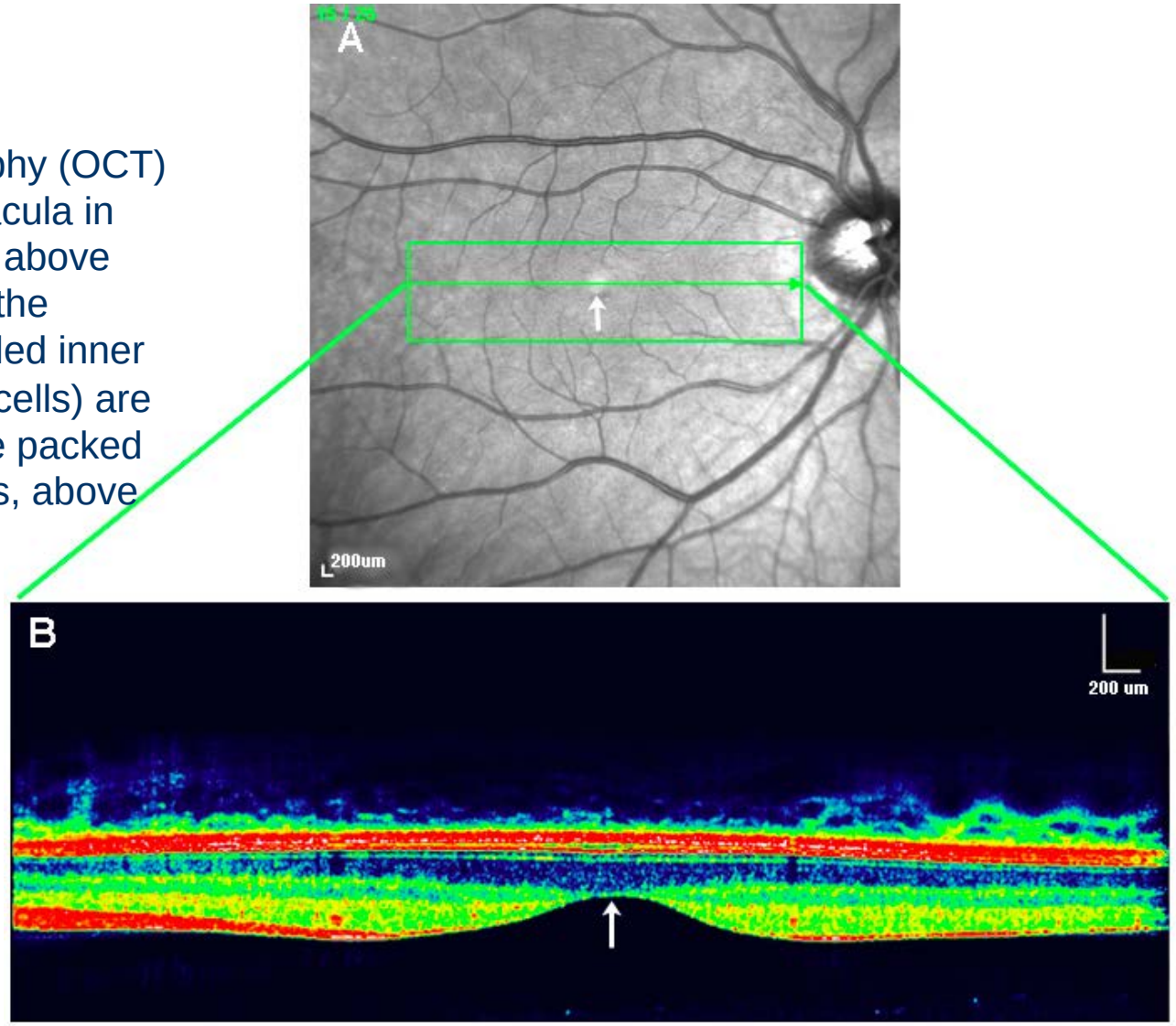


Os, outer segments
is, inner segments
OLM, outer limiting membrane
ONL, outer nuclear layer
H, Henle fibres
INL, inner nuclear layer
ILM, inner limiting membrane
G, ganglion cells

Foveal Centre (Pit)

A. Fundus photo of a normal human macula, optic nerve and blood vessels around the fovea.

B. Optical coherence tomography (OCT) images of the same normal macula in the area that is boxed in green above (A). The foveal pit (arrow) and the sloping foveal walls with dispelled inner retina neurons (green and red cells) are clearly seen. Blue cells are the packed photoreceptors, primarily cones, above the foveal centre (pit).



Phototransduction

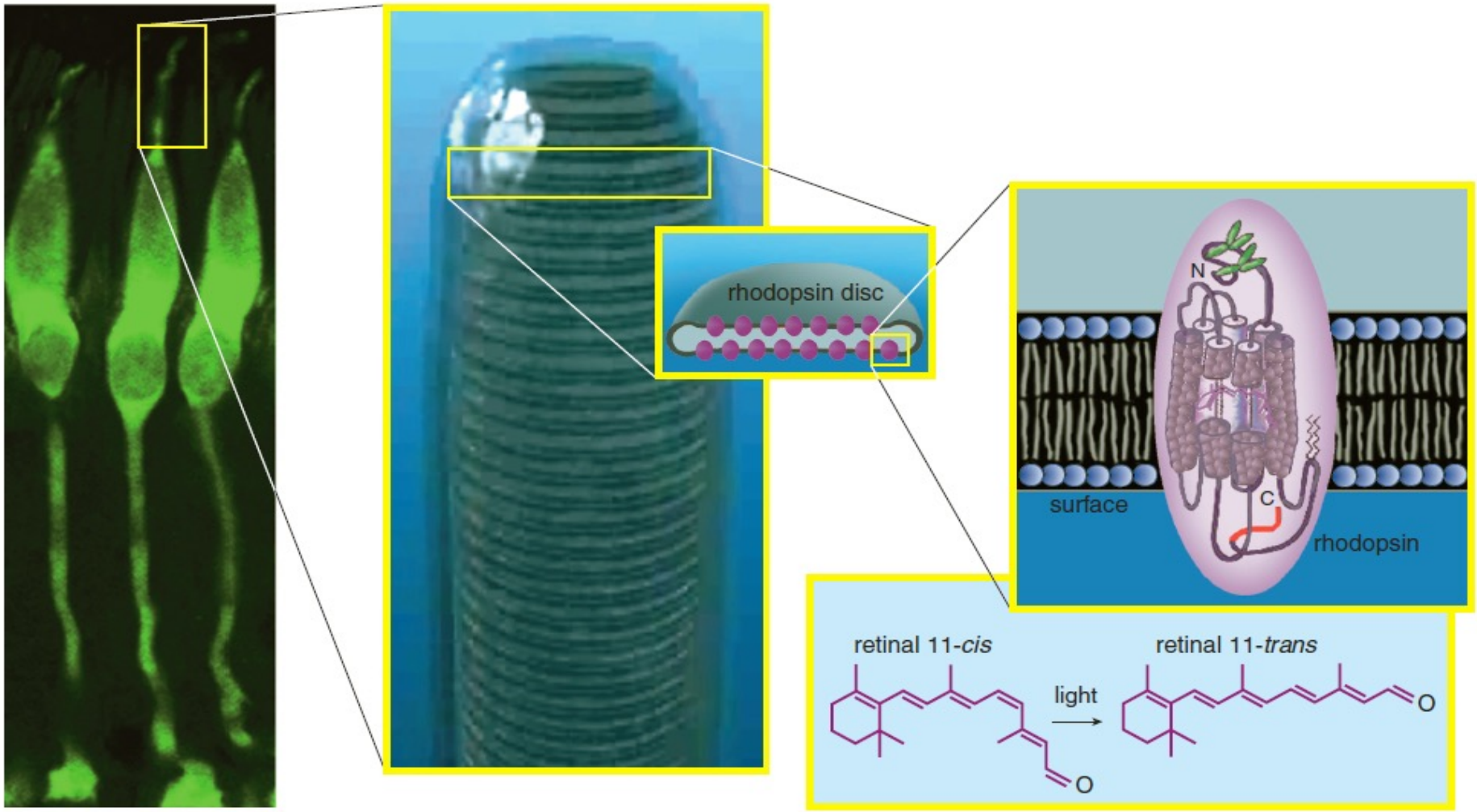
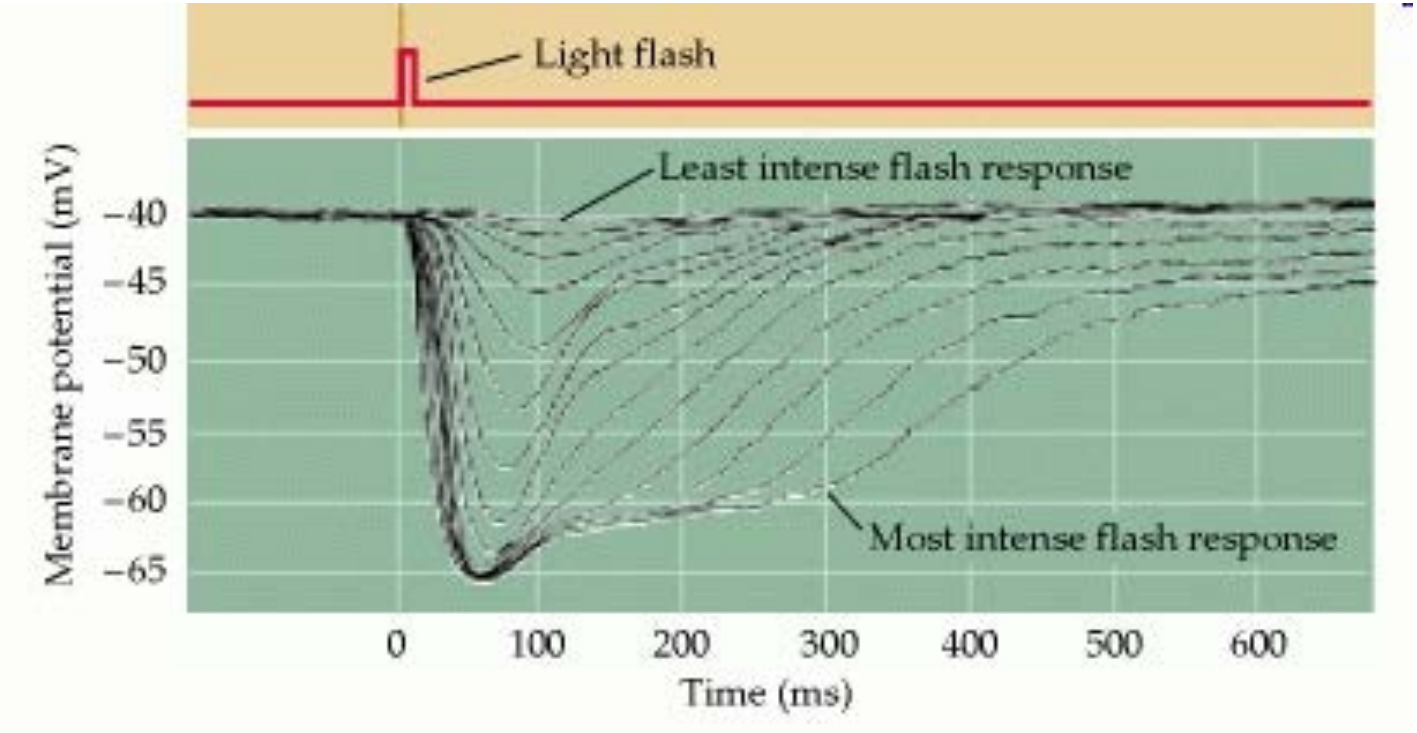


Figure 4. Cone photoreceptors from a monkey are stained with a fluorescent green dye *(left)*. When the outer segments of cones or rods are magnified further, stacked membrane disks are visible inside *(middle)*. The disks are studded with thousands of rhodopsin complexes. Each rhodopsin consists of a membrane-traversing protein with a retinal molecule embedded in its core *(right)*. When exposed to light, one of the bonds in the retinal molecule rotates, changing the shape of the protein *(lower right)*. (Middle photograph courtesy of Carlos Rozas.)

From: **Kolb, H.,** *American Scientist*, 91 (2003)

Photoreceptor responses

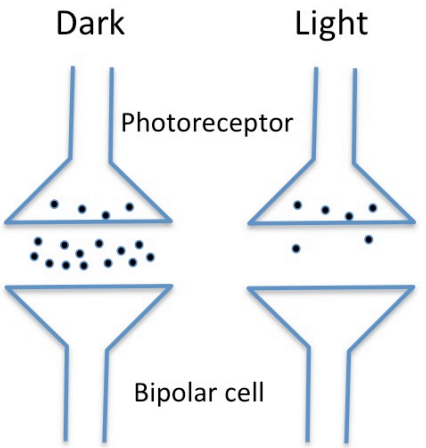


An intracellular recording from a single cone stimulated with different amounts of light (cone from the turtle retina).
Each trace represents the response to a brief flash that was varied in intensity.
At the highest light levels, the response amplitude saturates (at about -65 mV).
The hyperpolarizing response is characteristic of vertebrate photoreceptors.

(After Schnapf and Baylor, 1987.)

Photoreceptor response to light

- In the dark (*i.e.* absence of light, *a.k.a.* **OFF**), photoreceptors have a depolarised cell membrane potential and are releasing their neurotransmitter (L-glutamate) continuously.

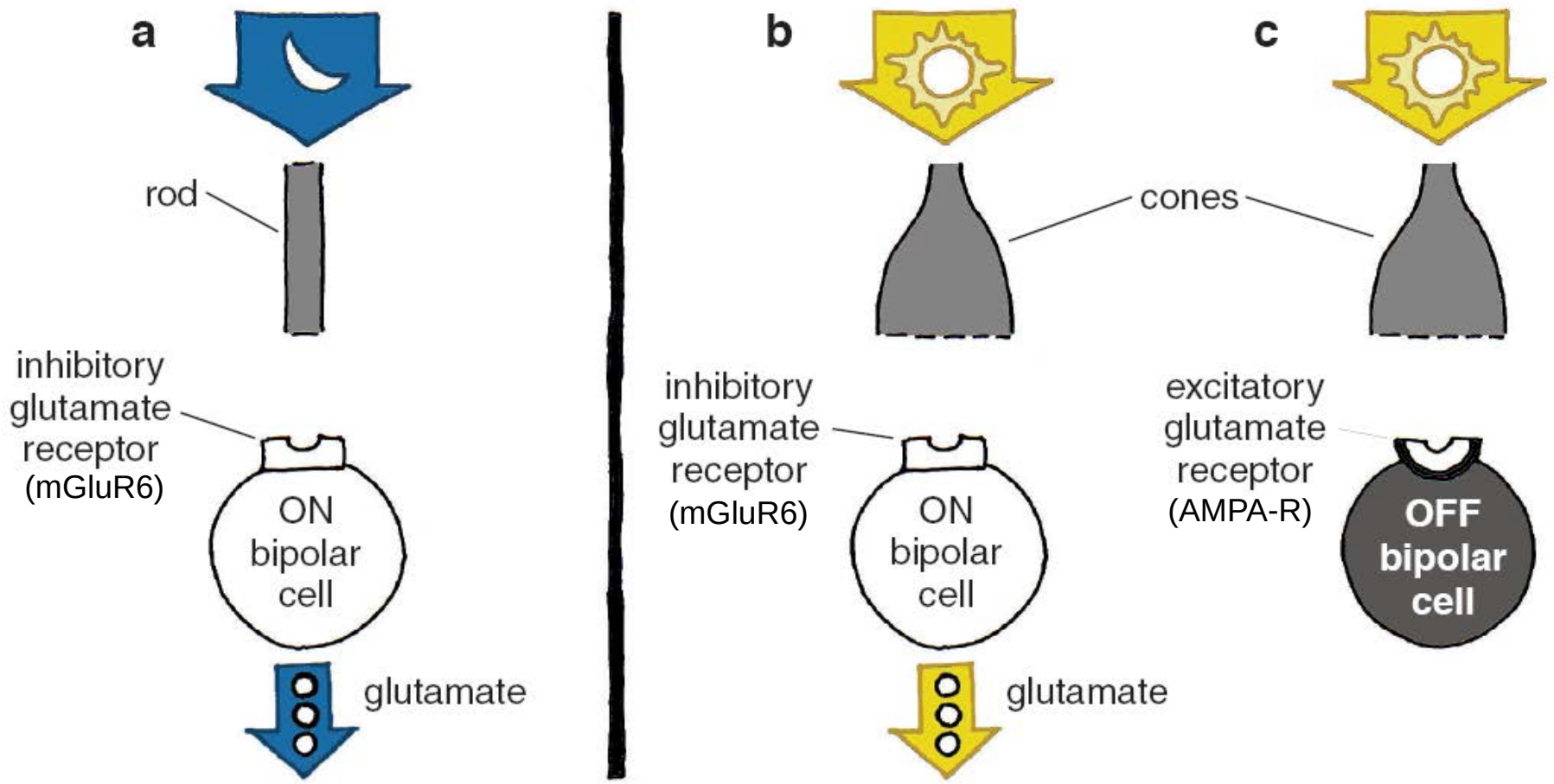


- Photoreceptors always respond to light ON with membrane potential hyperpolarisation: this results in a reduction of neurotransmitter release.

- Thus Photoreceptors can be considered to have an **OFF** response

- Photoreceptors synapse onto Bipolar Cells……

ON and OFF bipolar cells



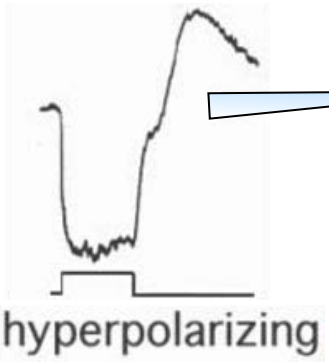
Photoreceptors release the transmitter glutamate in the dark, and stop releasing glutamate when stimulated by light.

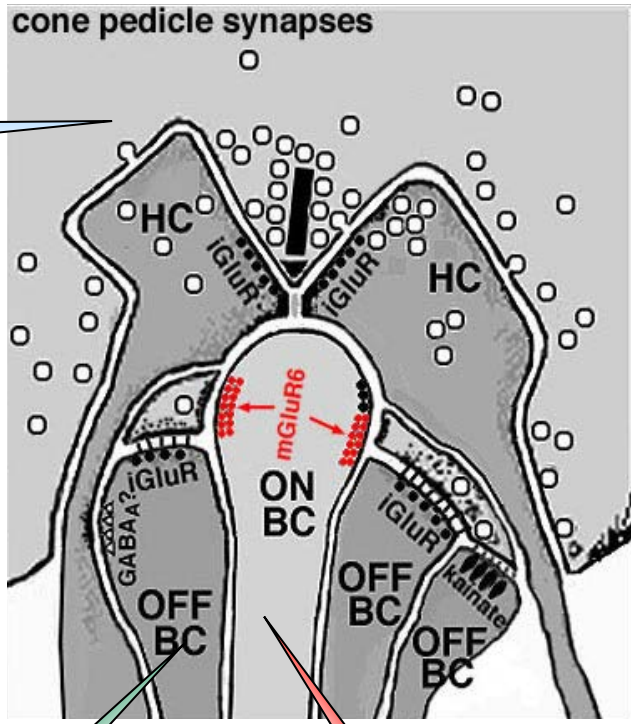
Different bipolar cells respond differently to glutamate, depending on their type of glutamate receptor:

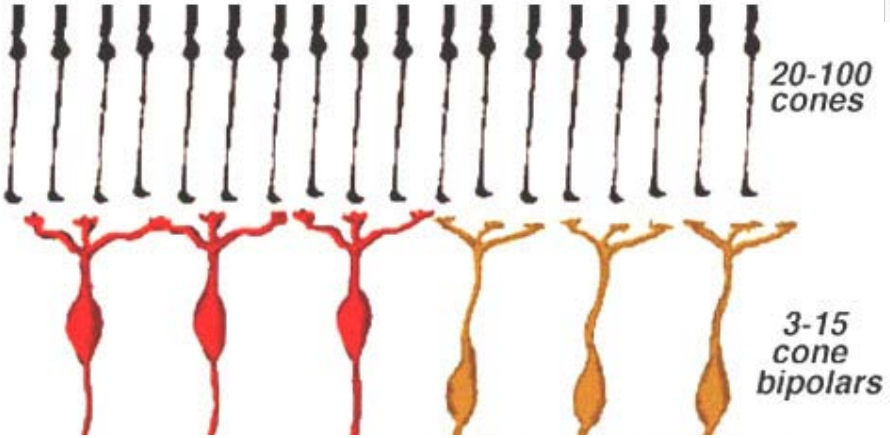
- ON bipolar cells have a depolarising receptive field (a, b)
- OFF bipolar cells have a hyperpolarising receptive field (c).

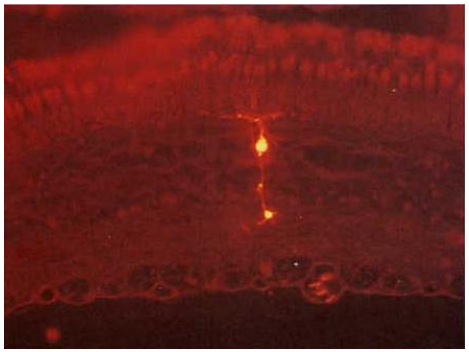
Cone Bipolar Cells

Connections of Cone photoreceptors
to ON- and OFF- Cone Bipolar Cells

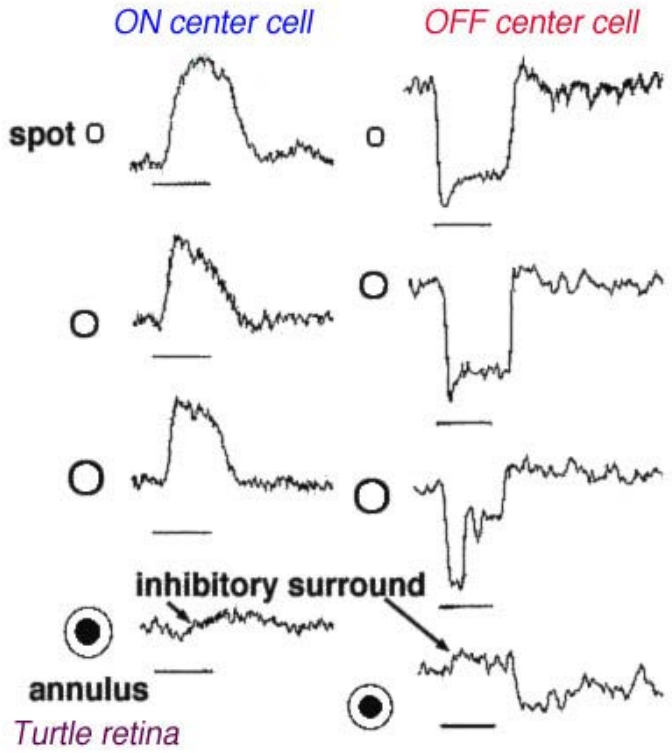




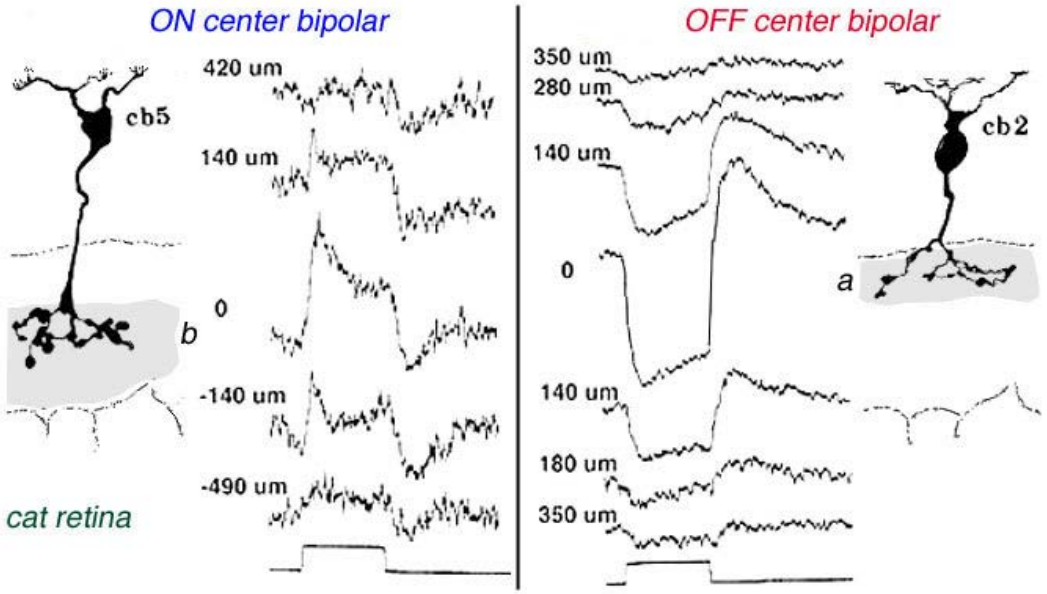




Bipolar Cell Responses

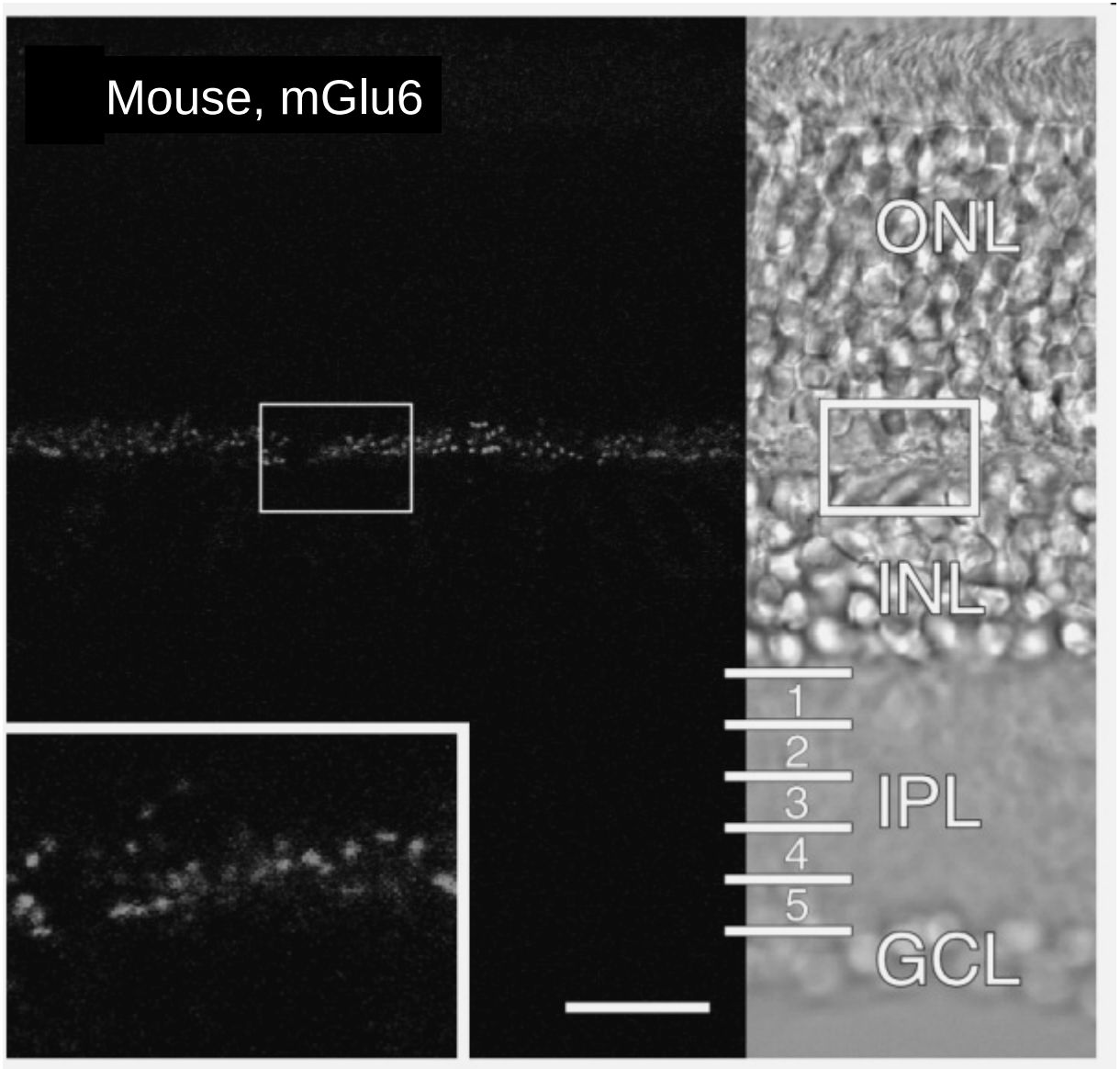


Intracellular recordings from two types of bipolar cells in turtle retina (Ammermuller and Kolb, 1995).



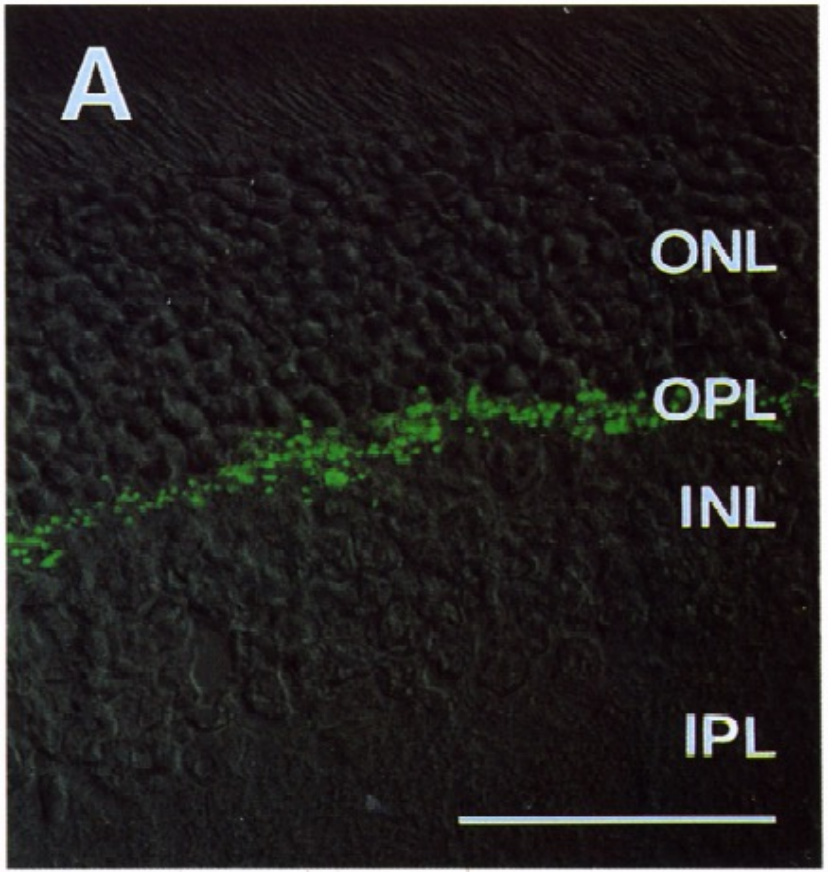
Intracellular recordings from ON and OFF bipolar cells in cat retina. Size of the receptive field measured in μm.

mGlu6 localisation in retina

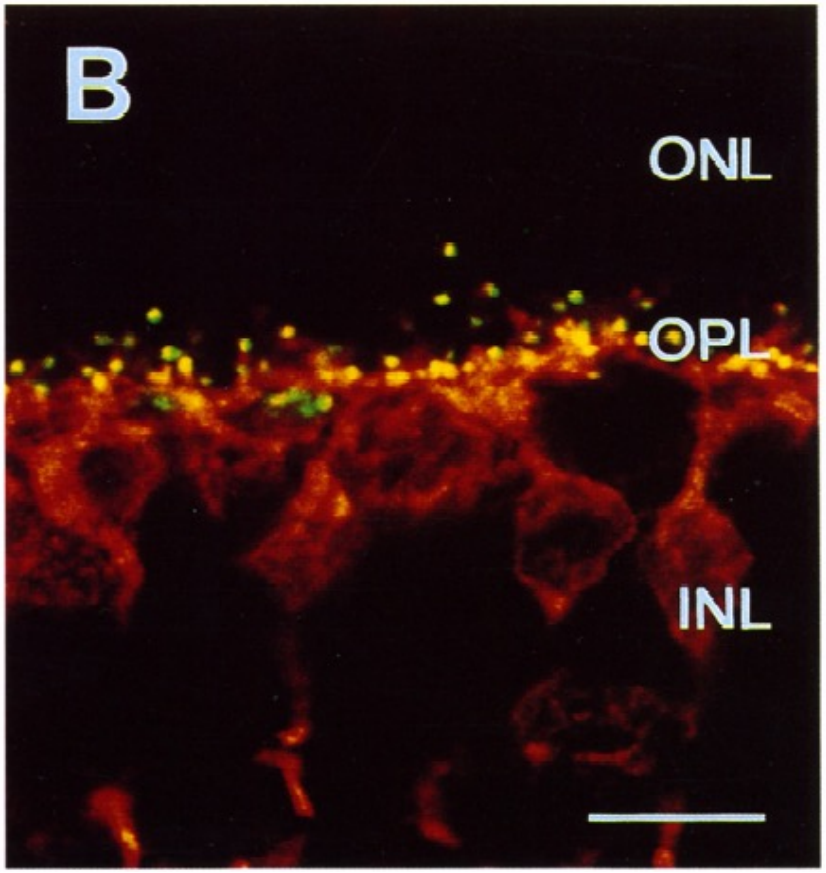


Quraishi S., Gayet J., Morgans C.W., and Duvoisin R.M. (2007)
Distribution of group-III metabotropic glutamate receptors in the retina. J Comp Neurol 501, 931-43.

mGlu6 in bipolar cells



(A) Vertical sections of the adult rat retina were immunostained with the mGluR6 antibody, and aconfocal micrograph superimposed on the Nomarski image of an immunostained section is indicated. A punctate labeling pattern (green) is restricted to the OPL. IPL, inner plexiform layer.



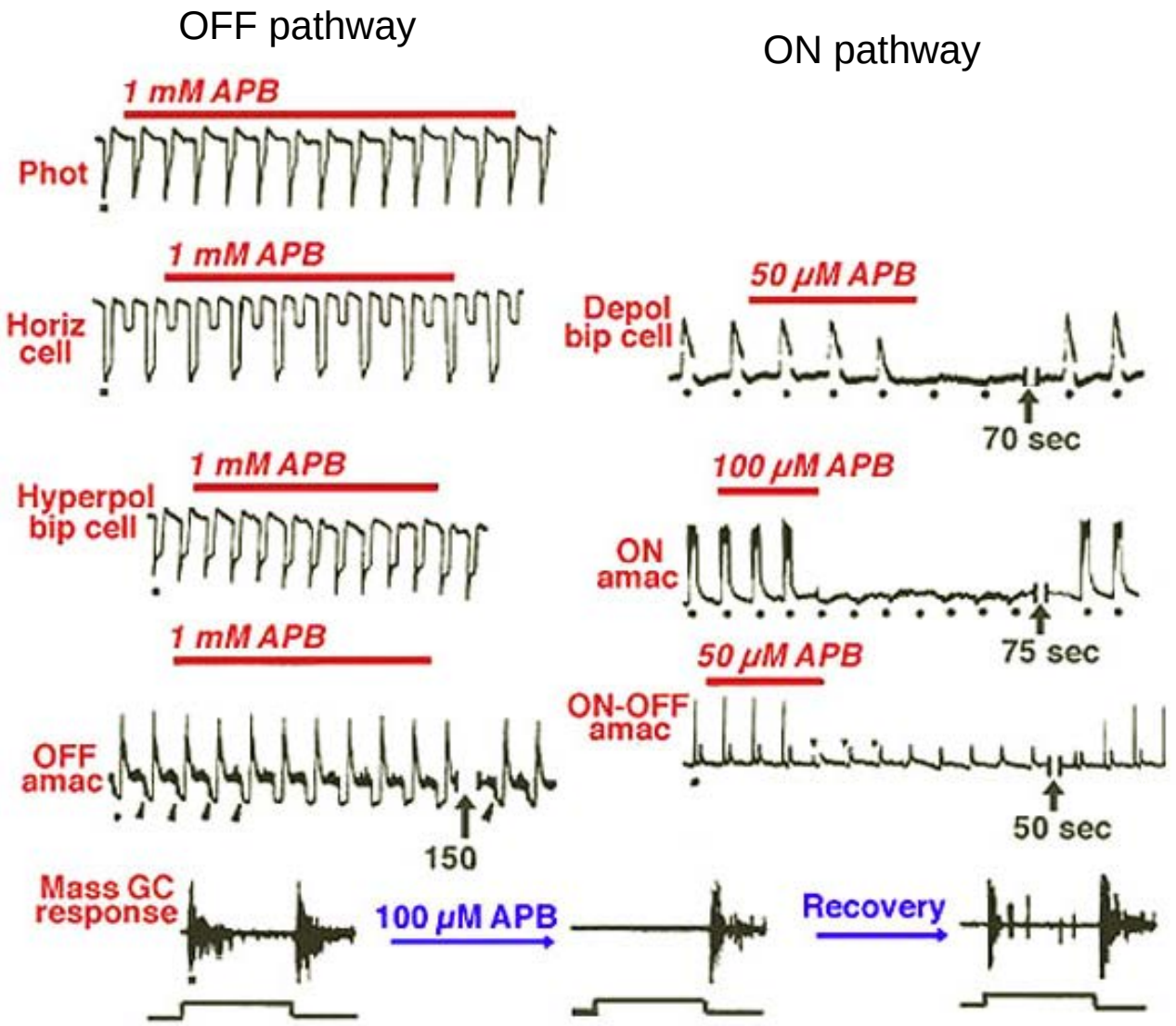
(B) Sections of the adult rat retina were immunostained with the mGluR6 antibody (green and yellow) and the PKC antibody(red), and a confocal micrograph of a double-stained section is presented. Intense punctate mGluR6 immunoreactivity is seen only at the dendritic termini of the PKC-immunoreactive bipolar cells.

Nomura *et.al.* (1994) Developmentally regulated postsynaptic localization of a metabotropic glutamate receptor in rat rod bipolar cells. Cell 77:361-369.

L-AP4 effects in the Retina

Note

- L-AP4 (formerly known as APB) is a Group III mGlu *AGONIST*.
- L-AP4 is a functional antagonist of the ON pathway.



intracellular recordings from different retinal neurons show that APB selectively antagonizes the ON pathway (right column). From Slaughter and Miller, 1981.

mGlu6 Receptor gated currents

Note

- mGlu6 is a Group III metabotropic glutamate receptor
- L-AP4 (formerly known as APB) is a Group III mGlu *AGONIST*.
- mGlu6 activation closes cation channels

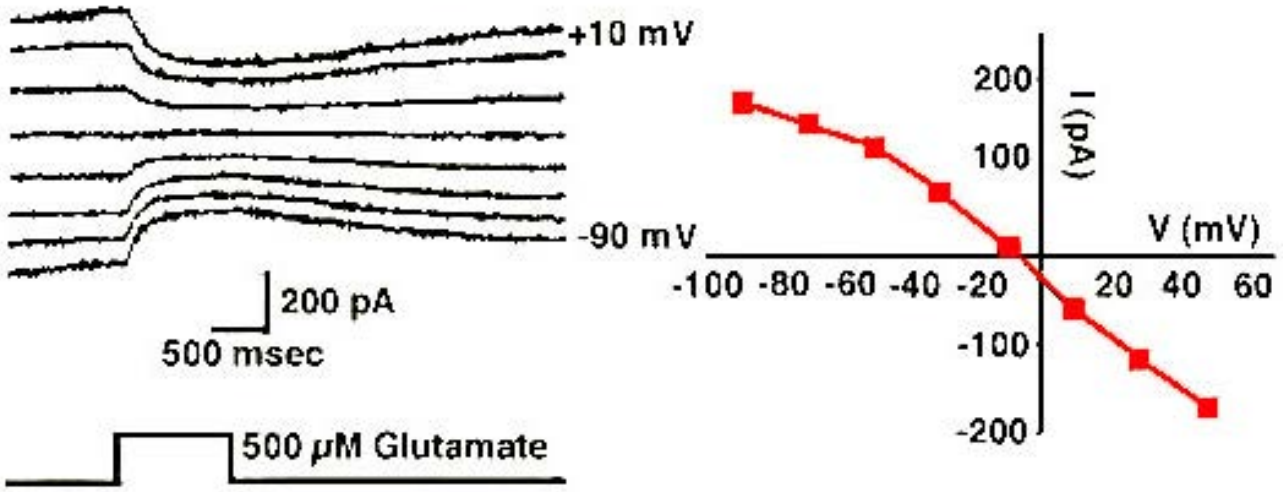


Fig. 10. Whole cell current traces (left) and the calculated current-voltage relationship (right) for APB receptor-gated currents. Glutamate acting on APB receptors closes non-selective cation channels resulting in a conductance decrease (from Grant and Dowling, 1996).

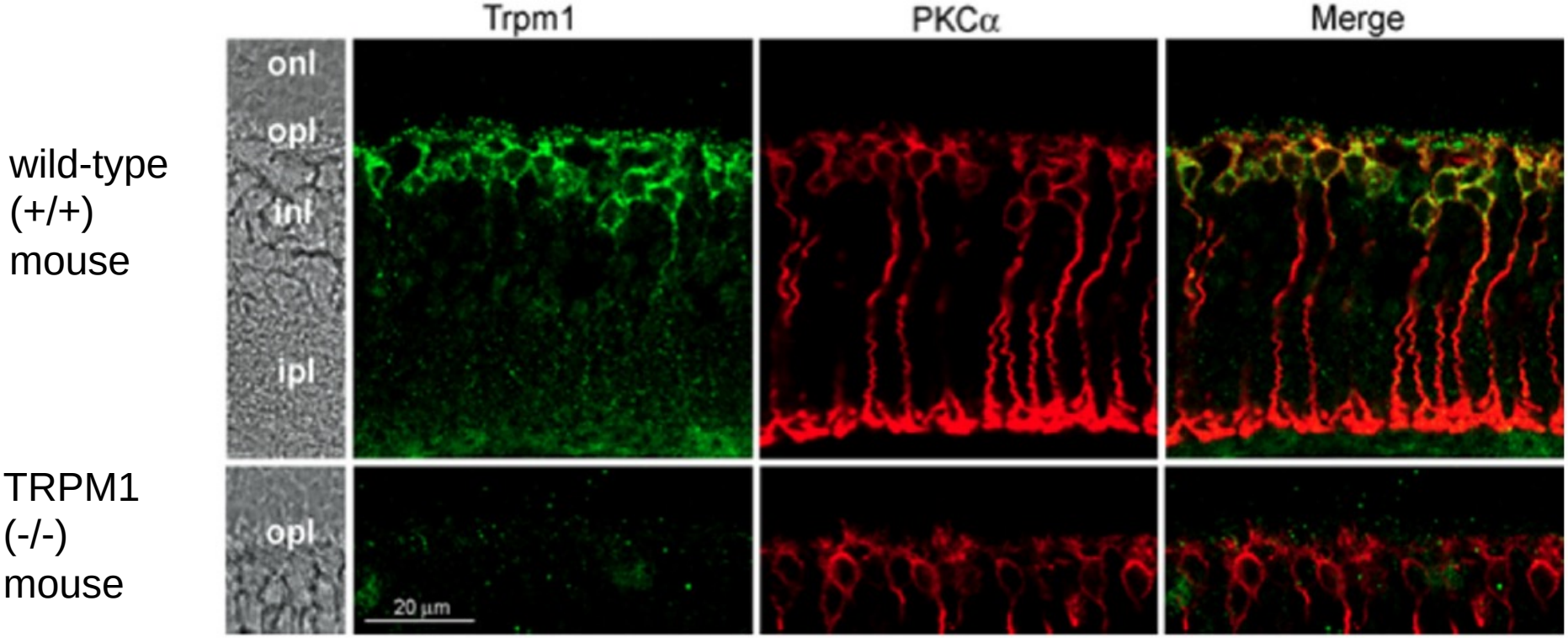
What is the Identity of the Cation Channel that mGluR6 gates/modulates?

- mGluR6-coupled current of ON-bipolar cells is inhibited by TRP* channel antagonists
- Congenital night blindness in Appaloosa horses linked to TRPM1 gene
- Electroretinograms on these horses indicate defective transmission between photoreceptors and ON-bipolar cells.

* Transient Receptor Potential

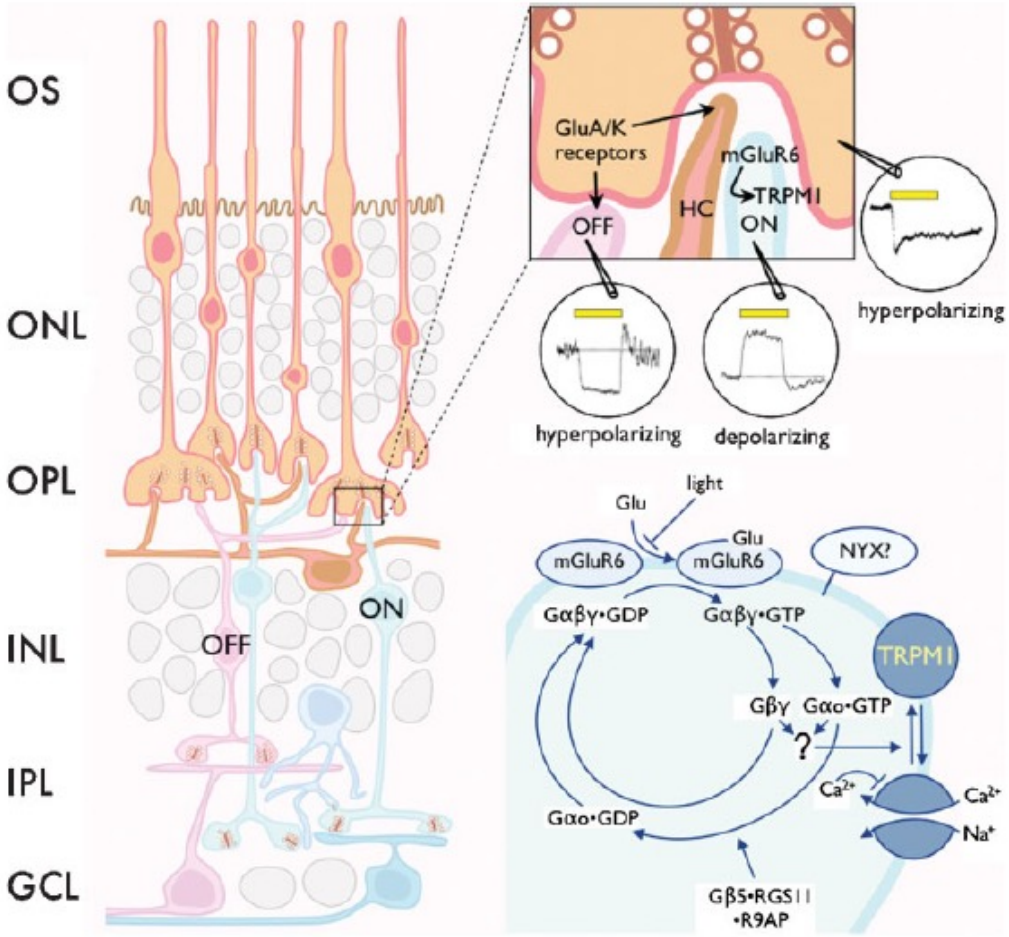
TRPM1 is expressed by ON-bipolar cells

Vertical sections from a wild-type (*Top*) and TRPM1-/- (*Bottom*) retina were immunofluorescently labeled by antibodies directed against TRPM1 (green) and PKC (red). Areas of colocalization appear yellow in the merged images



Morgans, C. W., *et al.* (2009). *Proc Natl Acad Sci U S A* **106**(45): 19174-19178.

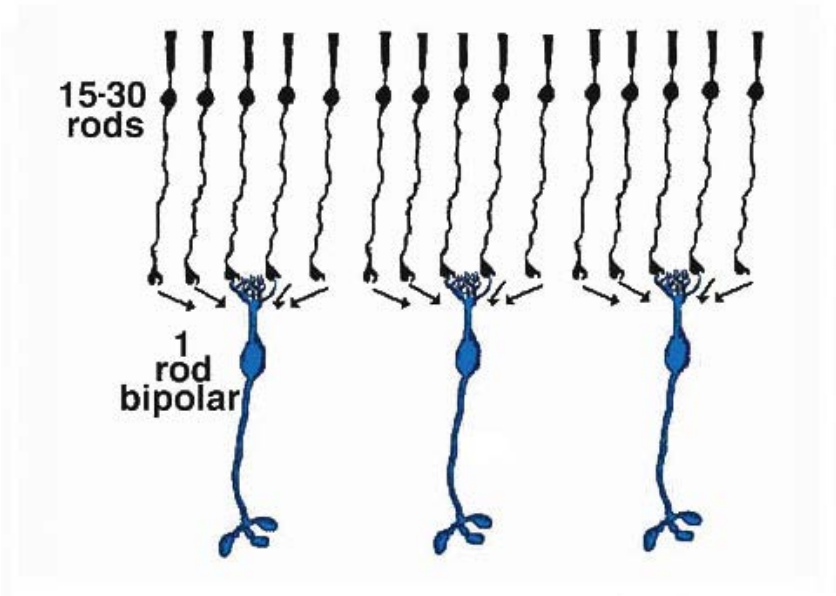
Summary: ON Bipolar Synapse



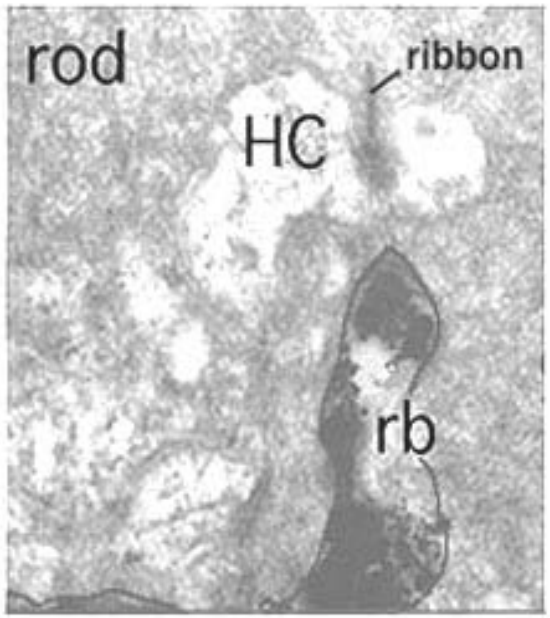
- All photoreceptors respond to light by hyperpolarization, as do horizontal cells (HC) and OFF bipolar cells (pink), which express AMPA and kainate-type glutamate receptors (GluA and GluK).
- ON-bipolar cells depolarize in response to light, a mechanism involving mGluR6 and TRPM1 channels.
- The G protein signaling cascade activated by mGluR6 is represented at the bottom right.
- The function of nyctalopin (NYX) and the second messenger signal between Go and the TRPM1 channel remain unknown.

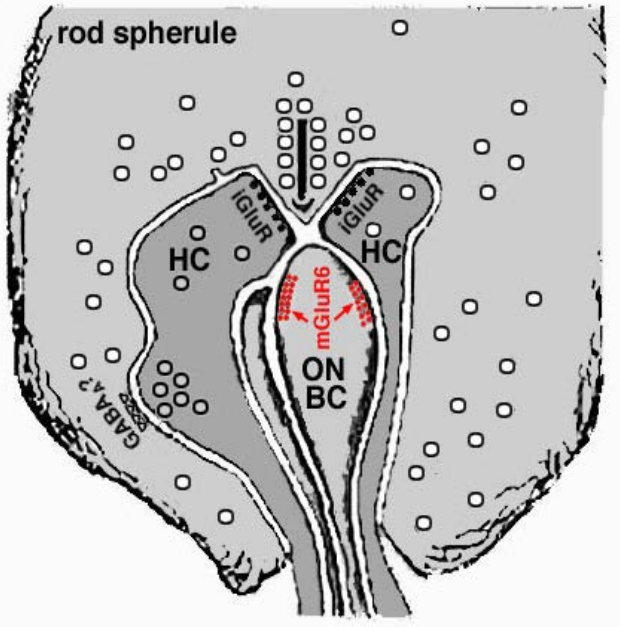
Morgans, C. W., et al. (2010). Bioessays 32(7): 609-614.

Rod pathway - Bipolar Cells



Convergence of rods onto rod bipolars

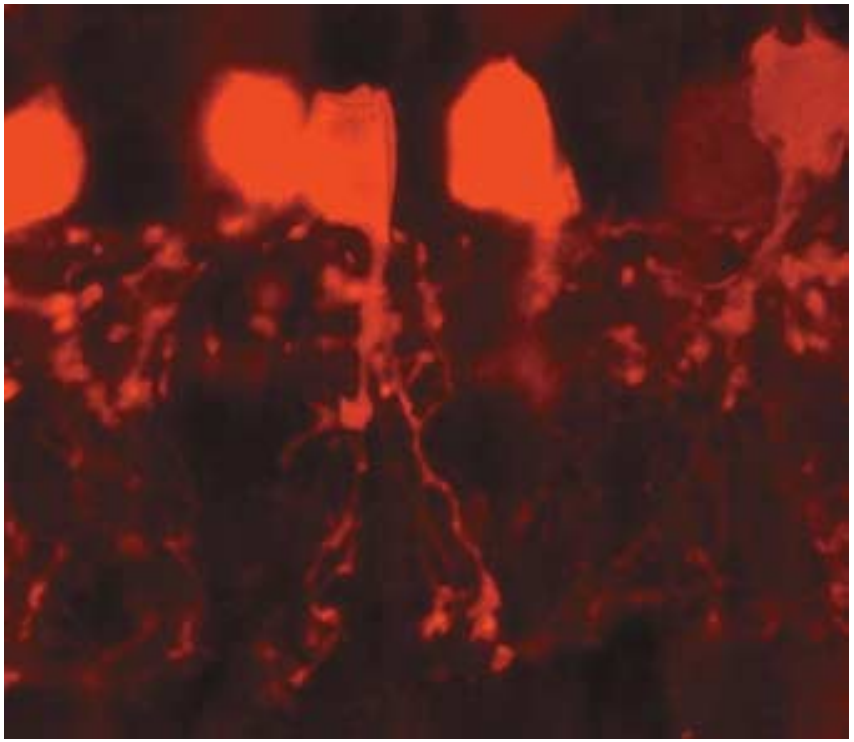


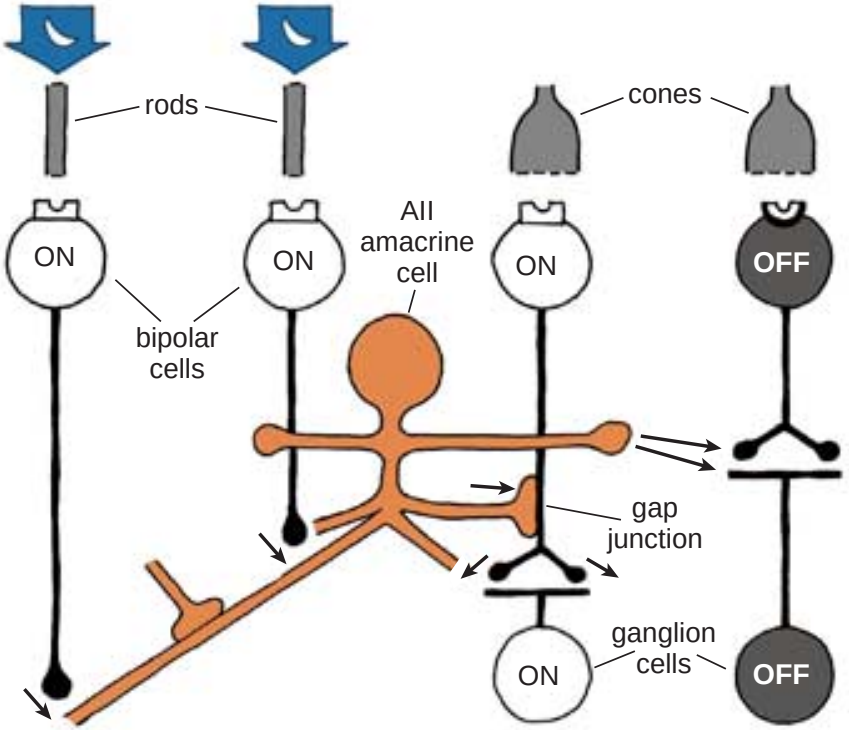


Electron micrograph and schematic of a rod spherule

BC – Bipolar Cell
HC – Horizontal Cell

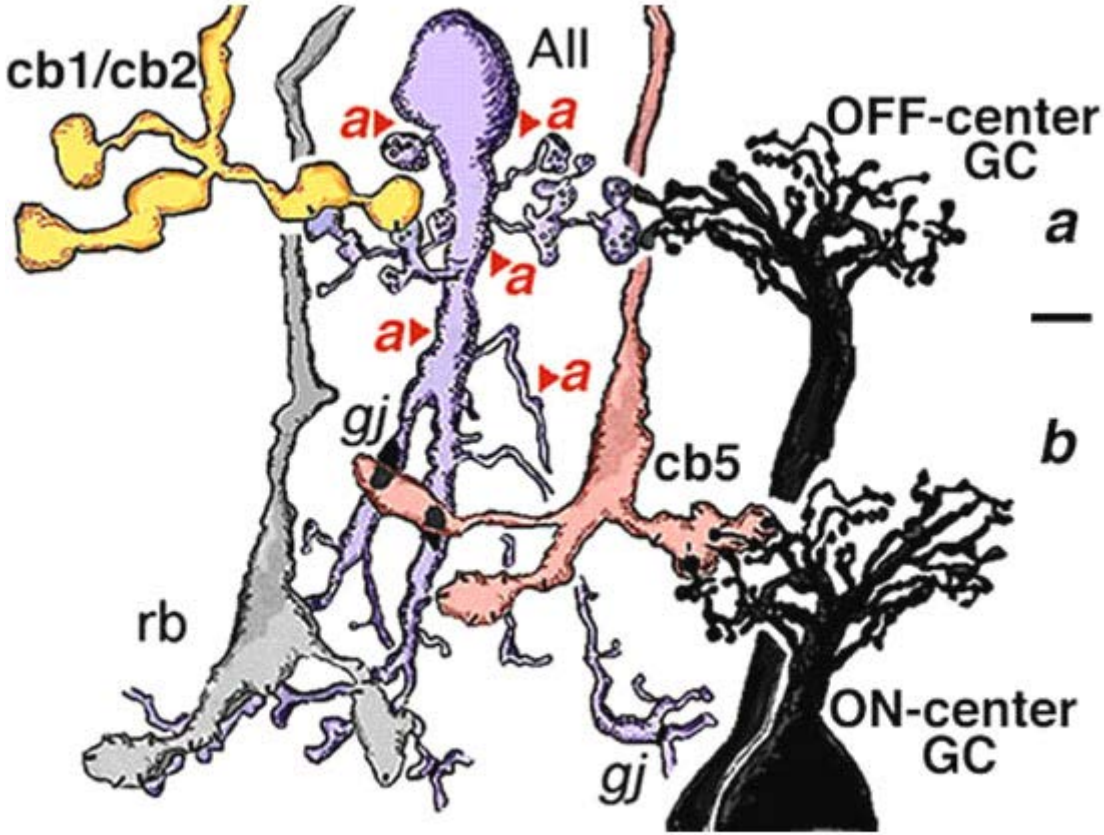
Rod pathway – The AII amacrine cell



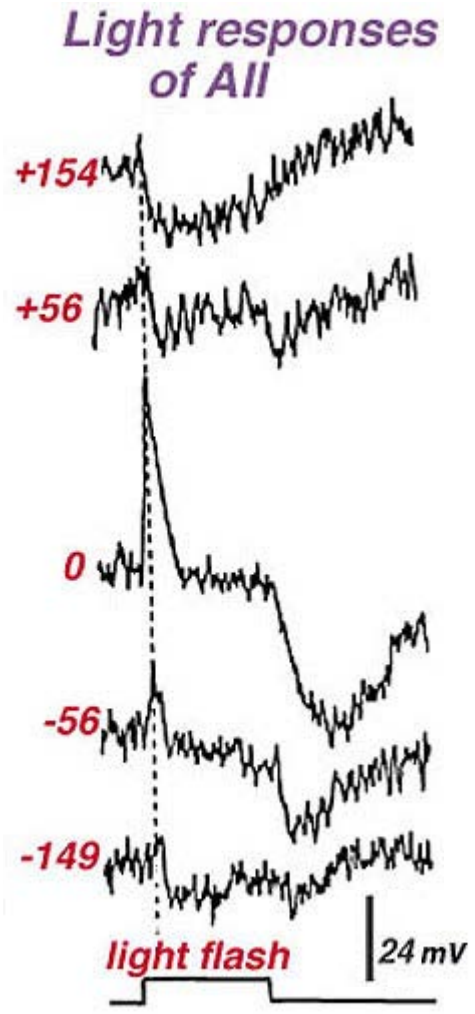


Rod bipolar cells communicate with ganglion cells indirectly using AII amacrine cells. The AII amacrine cells increase the signal under dim lighting conditions by coupling electrically to ON cone bipolar cells (gap junctions) and signalling chemically to OFF cone bipolar cells.

Amacrine Cell Function (AII)

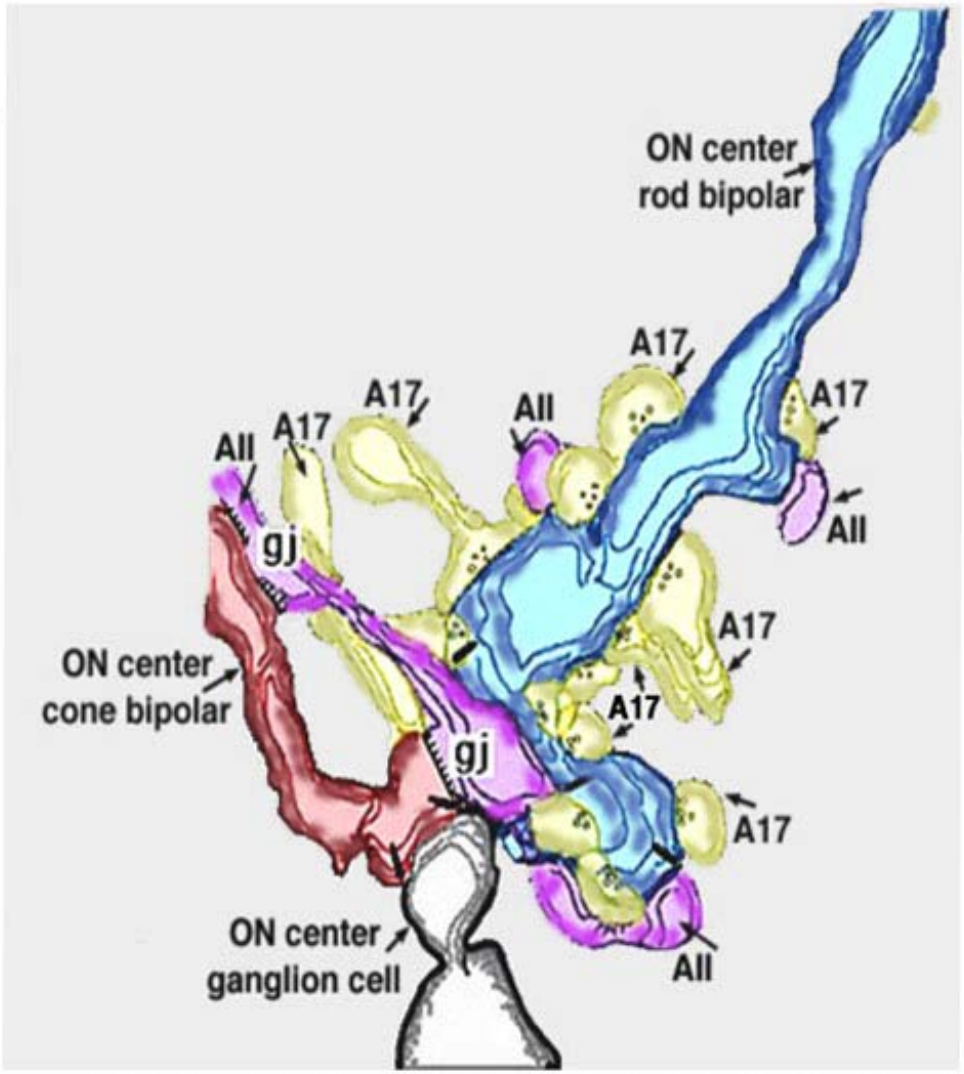


Drawing to show the circuitry of the AII amacrine cell with pre and post synaptic neurons. Sublaminas a and b are indicated.

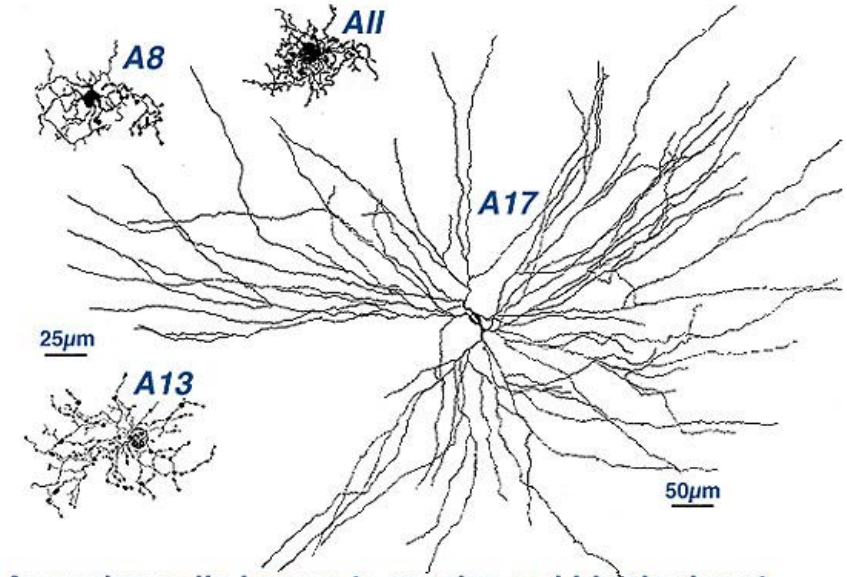


Intracellular recordings of AII amacrine cells. AII amacrine cells respond to light with a de-polarizing response (ON center response) in their centers (Nelson, 1982; Dacheux and Raviola, 1986).

Rods contact other amacrine cells



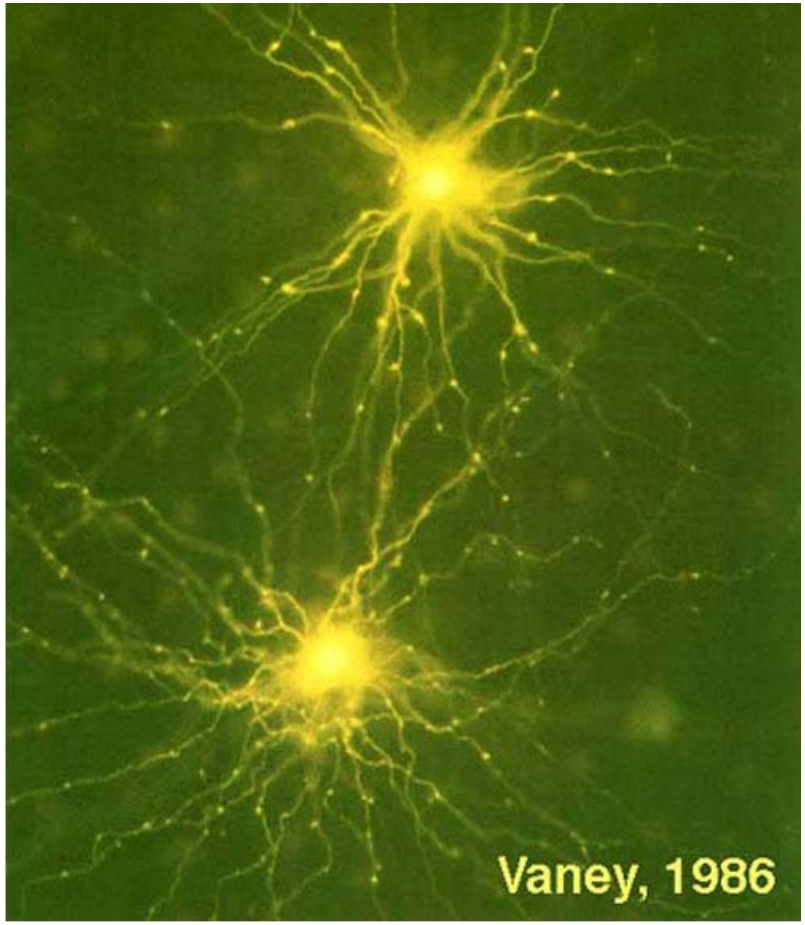
3-D reconstruction from serial electron micrographs of a rod bipolar axon terminal (blue) synapsing upon AII amacrine cell (lilac) and A17 amacrine cell (yellow) profiles. A17 processes make reciprocal synapses. AII amacrine cells make gap junctions on ON center cone bipolar axons.

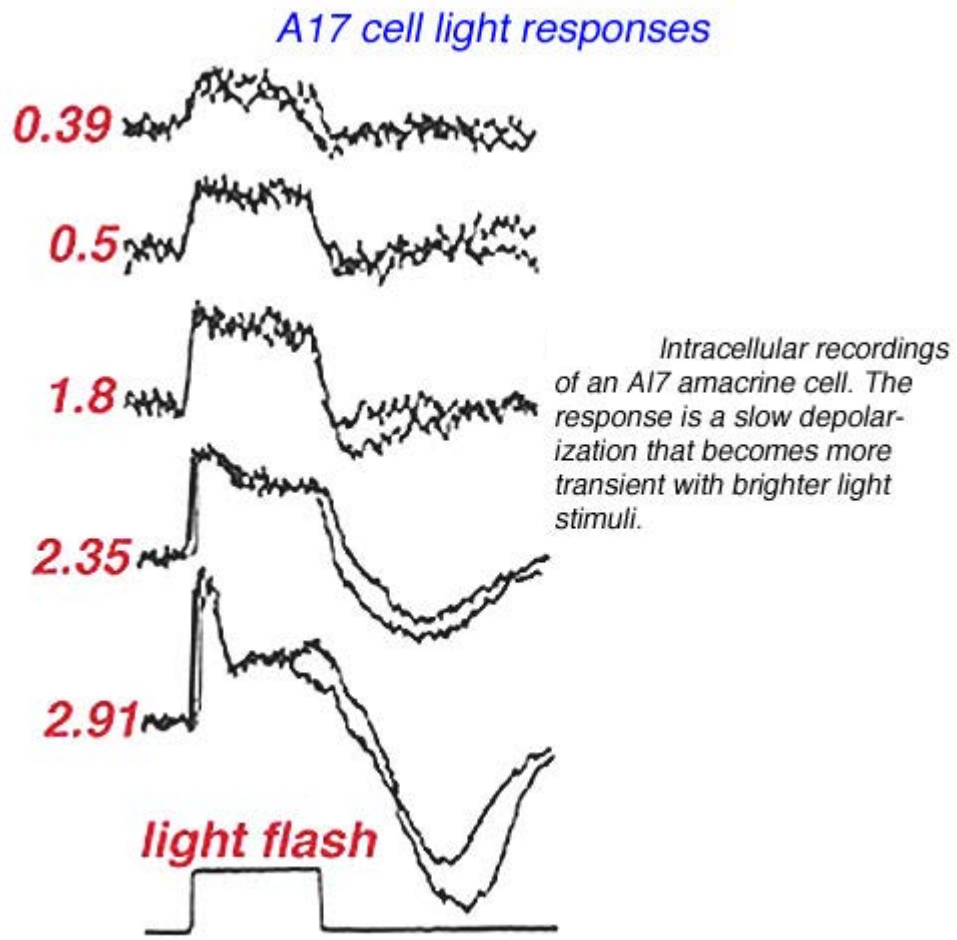


Amacrine cells known to receive rod bipolar input

There are ~22 different types of amacrine cell in the mammalian retina

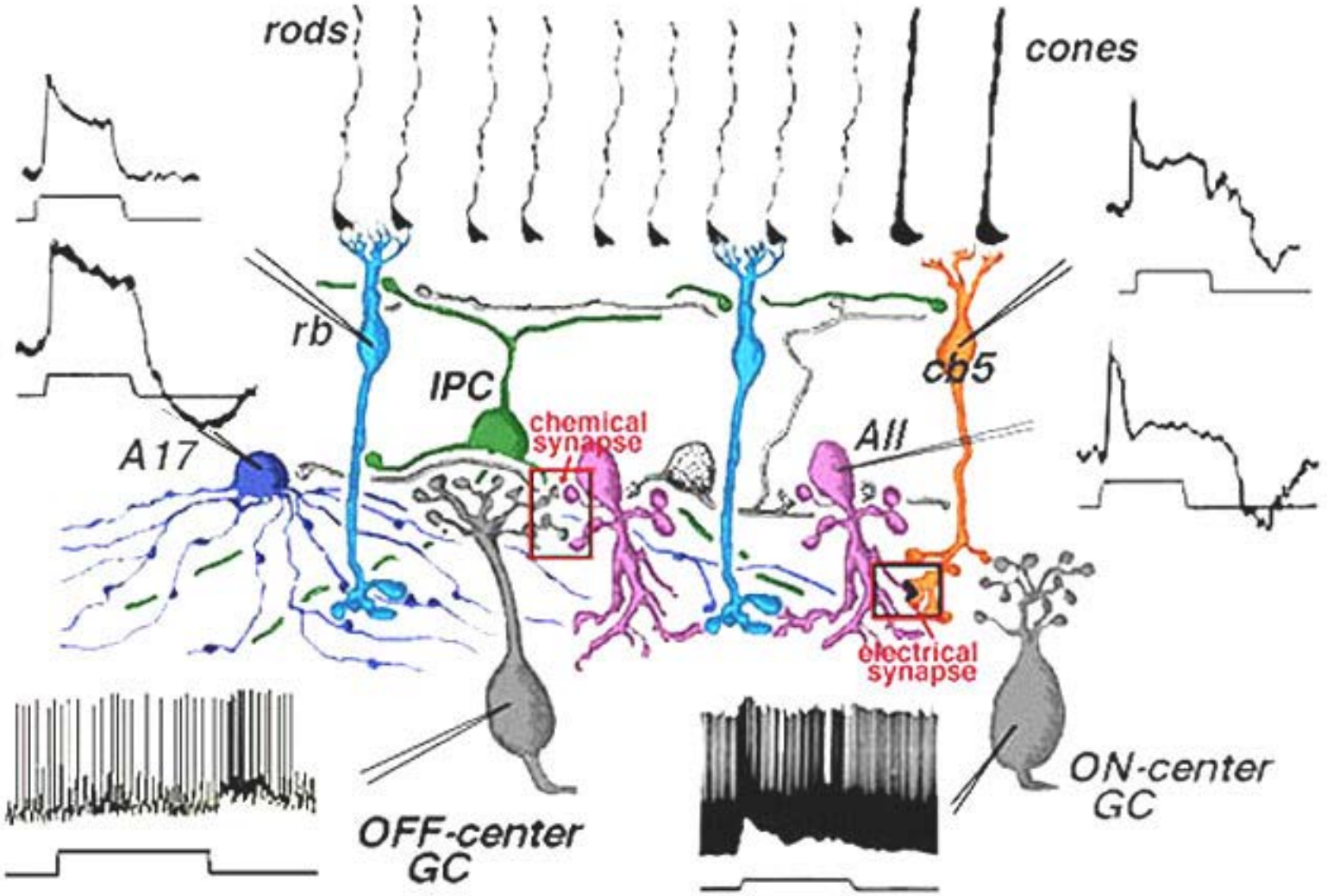
Amacrine Cell Function (A17)



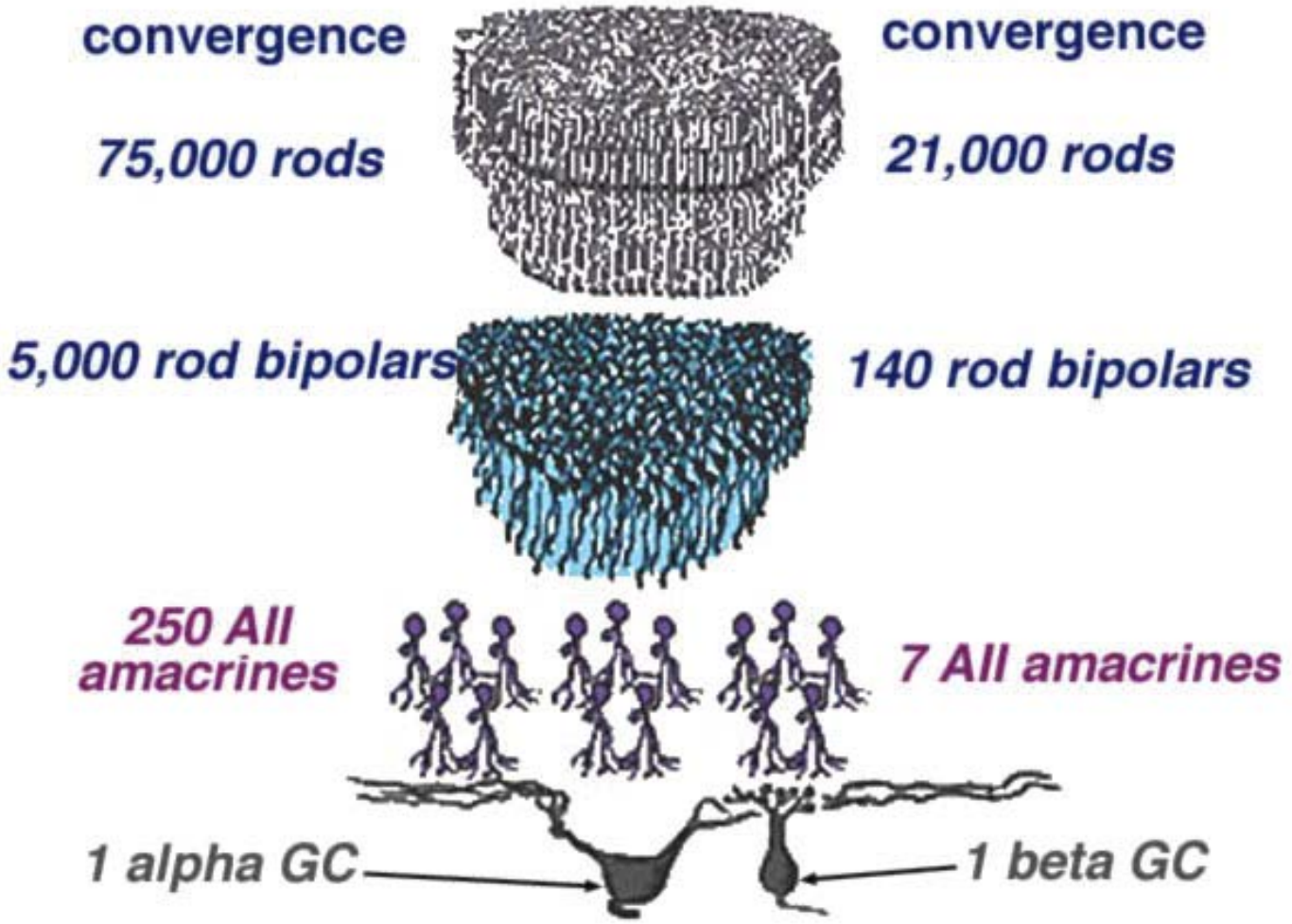


Intracellular recordings of an AI7 amacrine cell. The response is a slow depolarization that becomes more transient with brighter light stimuli.

Summary of Rod-driven pathways



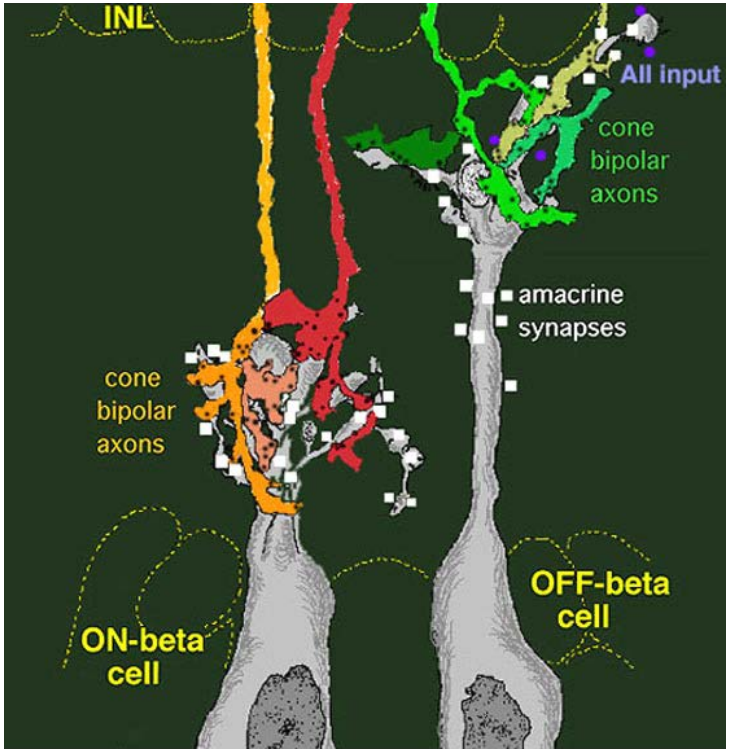
Convergence of the Rod Pathway

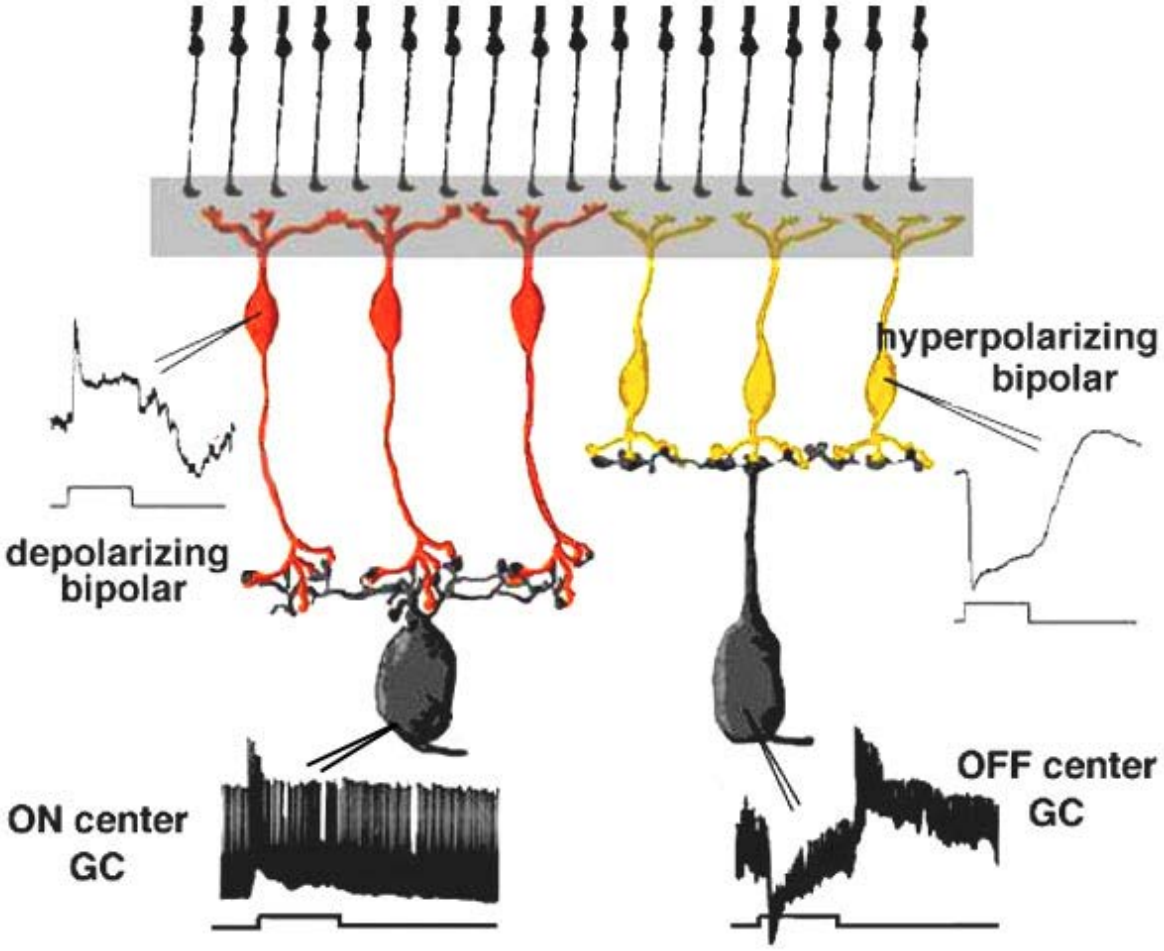


There are ~125 million photoreceptors (rods+cones) and only ~1 million retinal ganglion cells (GC).

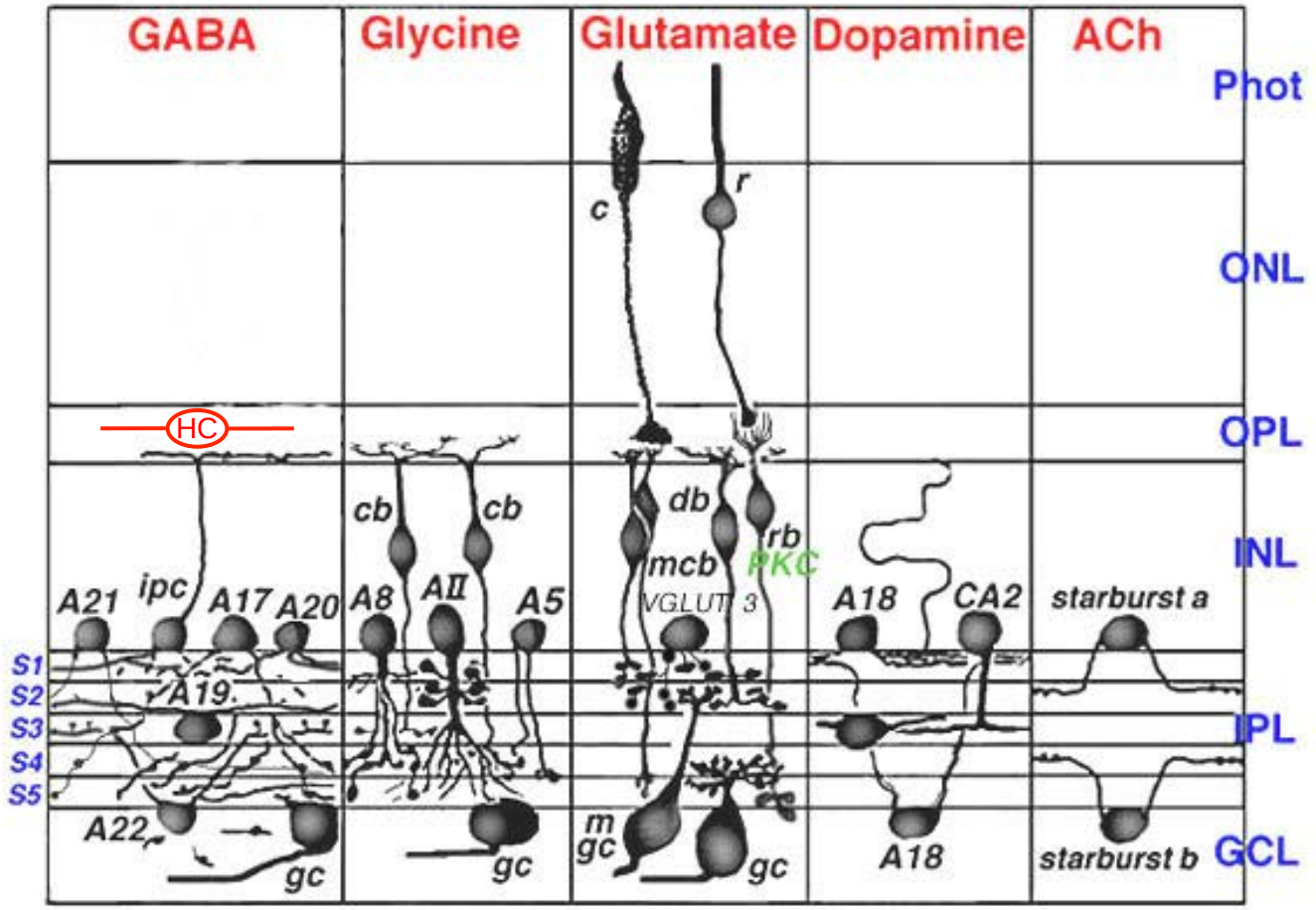
Cat retina

Cone Bipolar Termination on RGCs





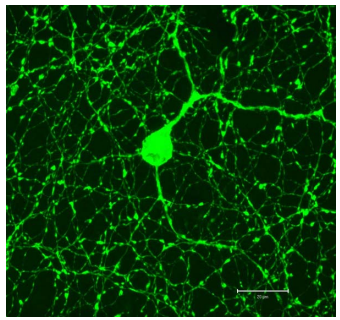
Neurotransmitters in Retina

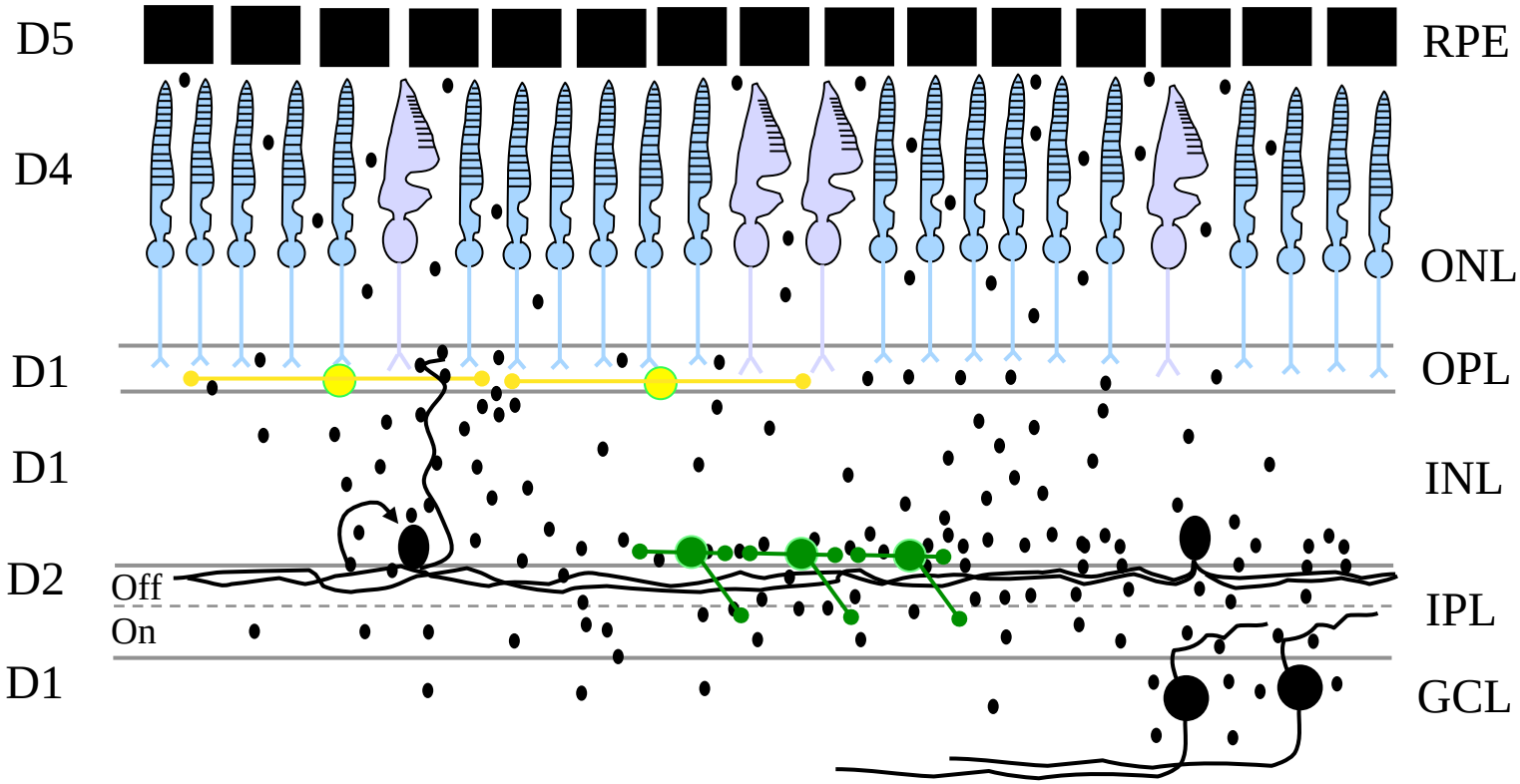


Organisation of neurotransmitters according to cell type in mammalian retina

Dopaminergic amacrine cells

Light adaptation of retinal circuitry by reducing gap junction coupling between horizontal and amacrine cells

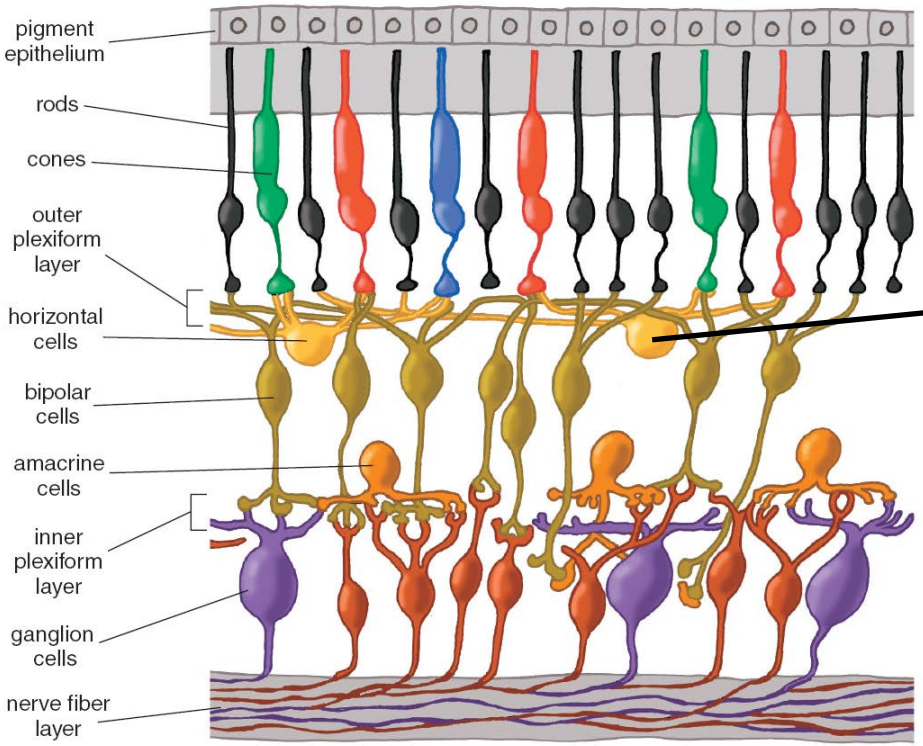


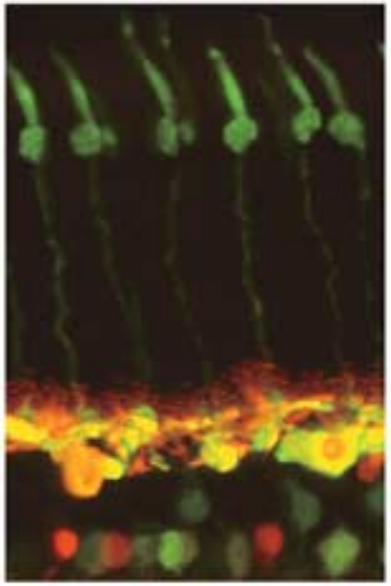


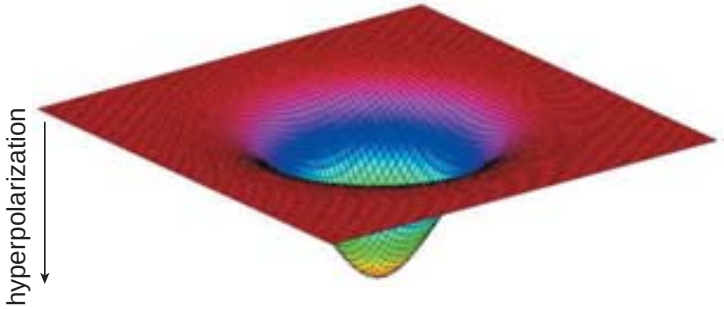
D1-D5 = distribution of dopamine receptors
Yellow: Horizontal cells, Green: Amacrine cells

Nitric Oxide (from different amacrine cells also reduces coupling between amacrines

Horizontal cells

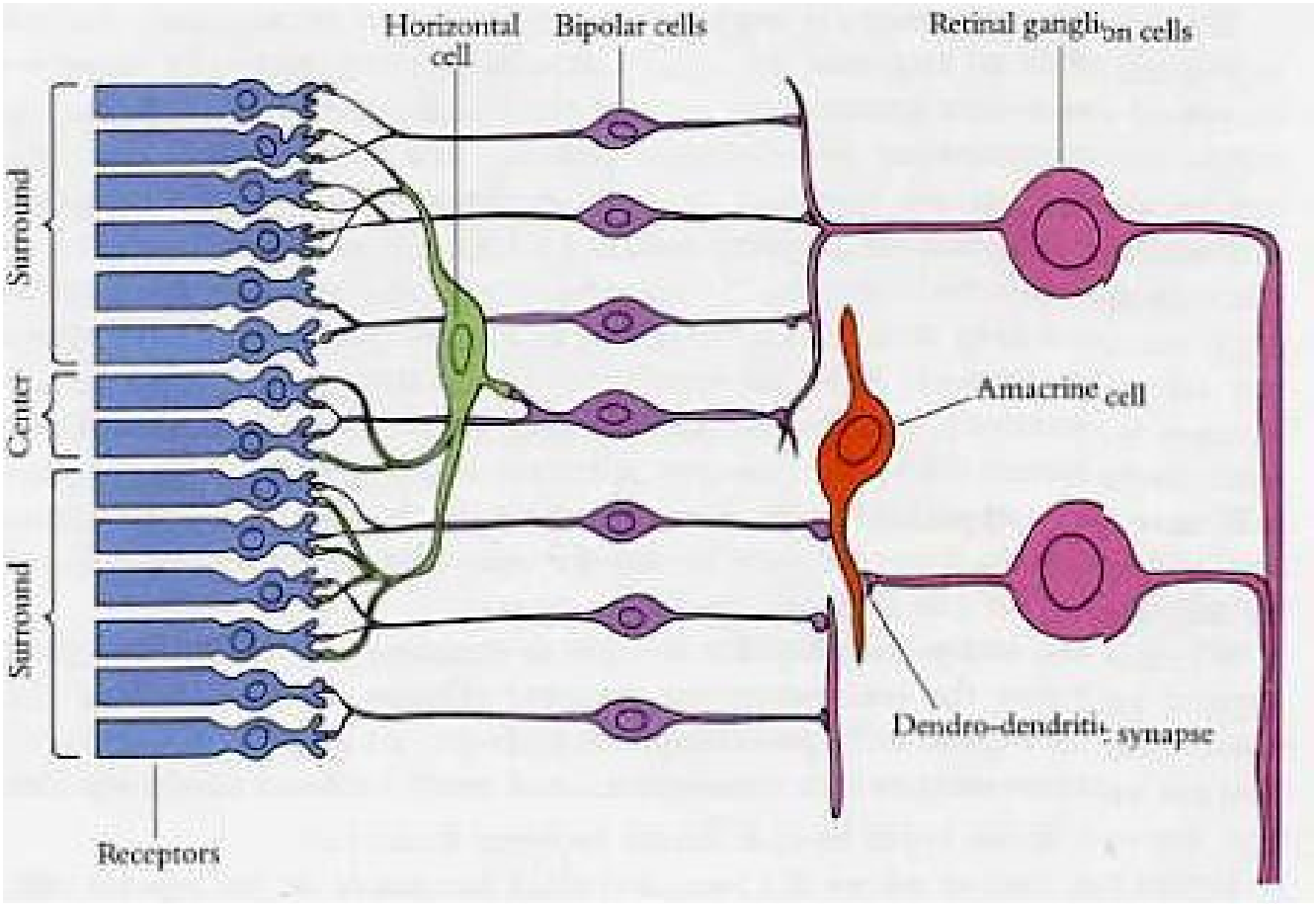




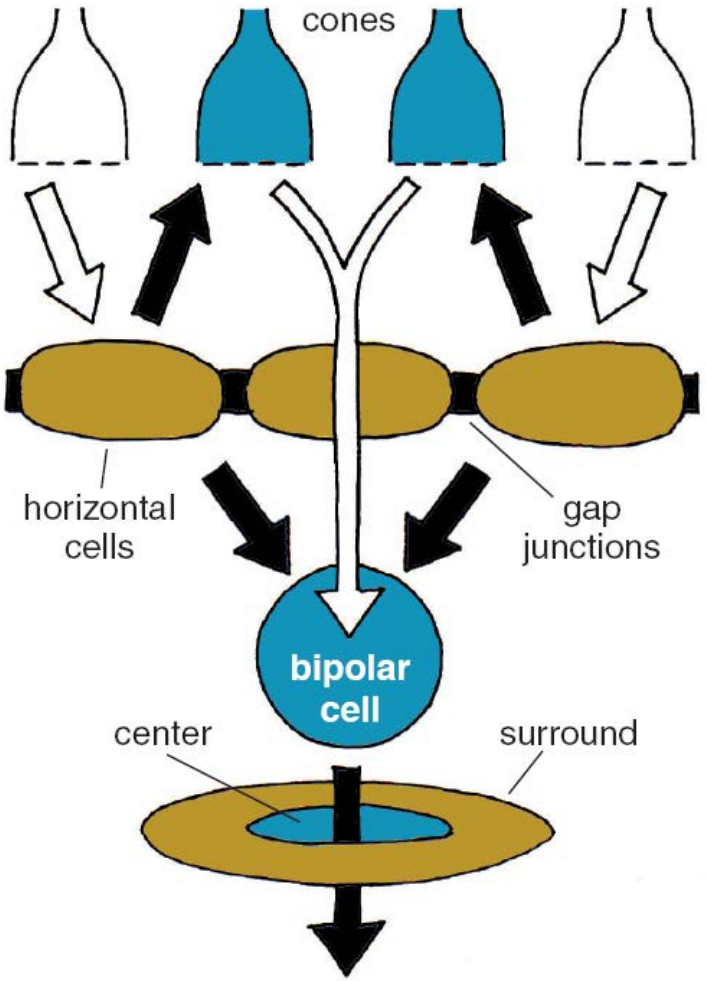


Horizontal cells hyperpolarize in response to light

Horizontal cells & lateral inhibition

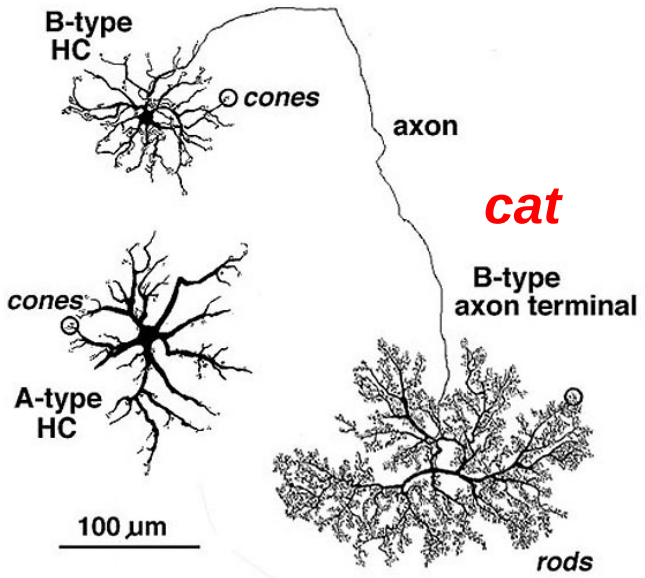


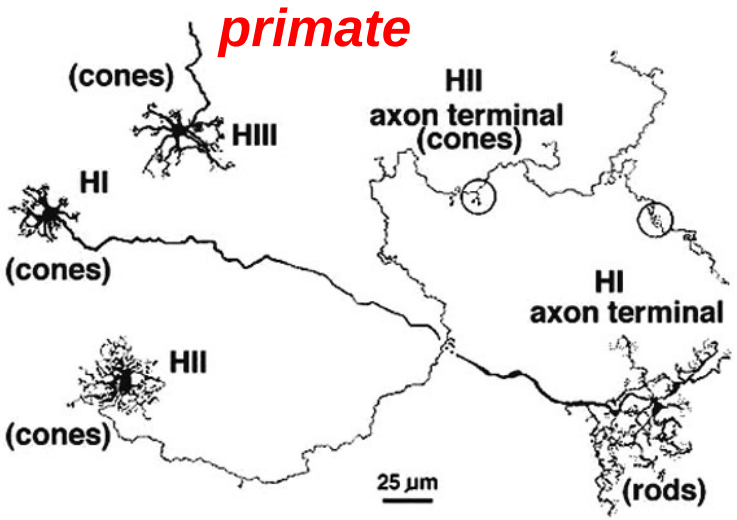
Horizontal Cells & lateral inhibition

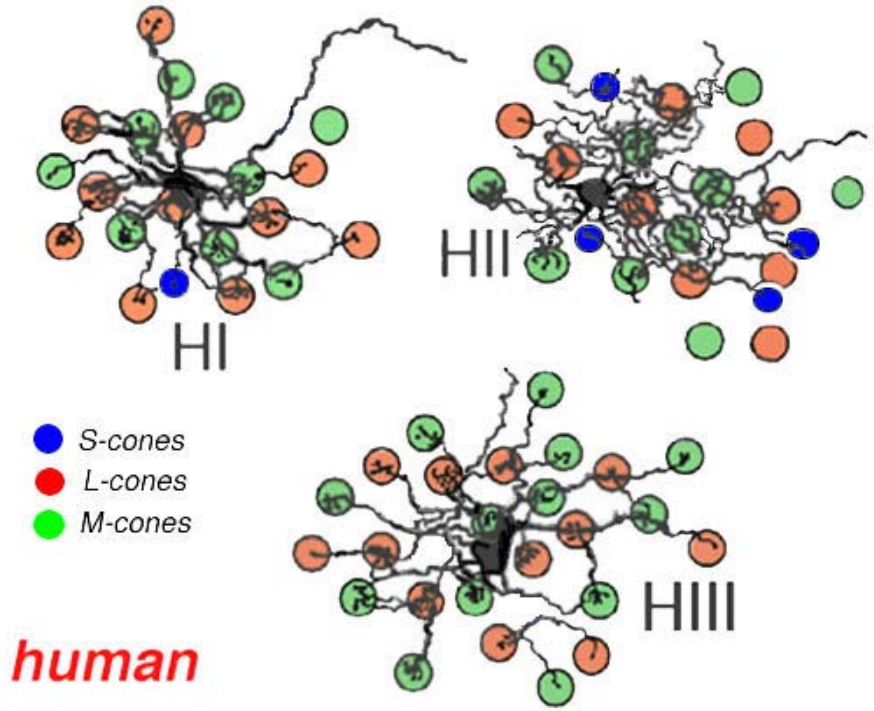


Horizontal cells receive information from many cones and influence bipolar cell signalling by adding an opponent (antagonistic) surround signal to the bipolar cells receptive field. Horizontal cells influence bipolar cells either directly or by feeding back Information to cones… probably both!

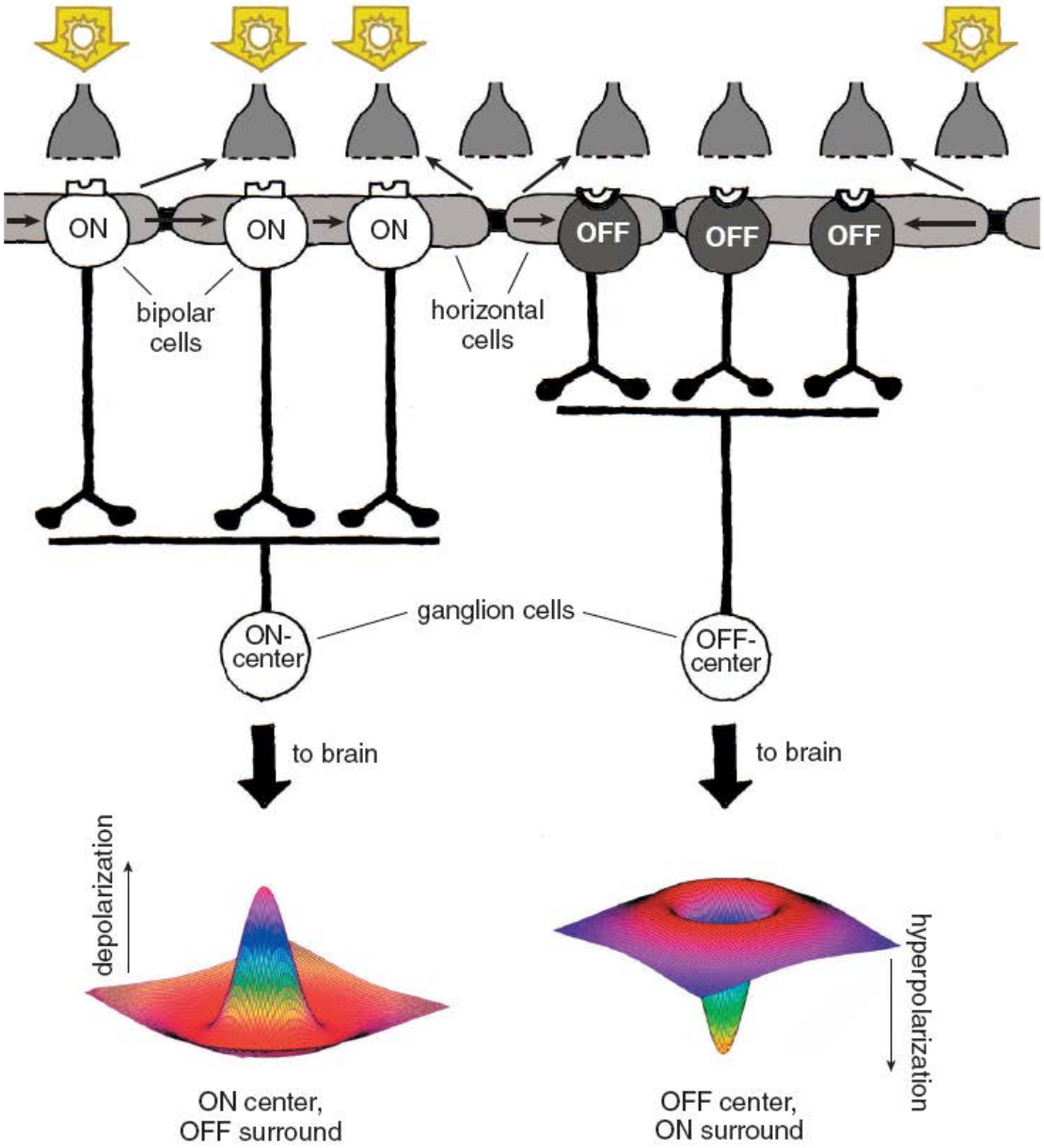
Horizontal Cell Types



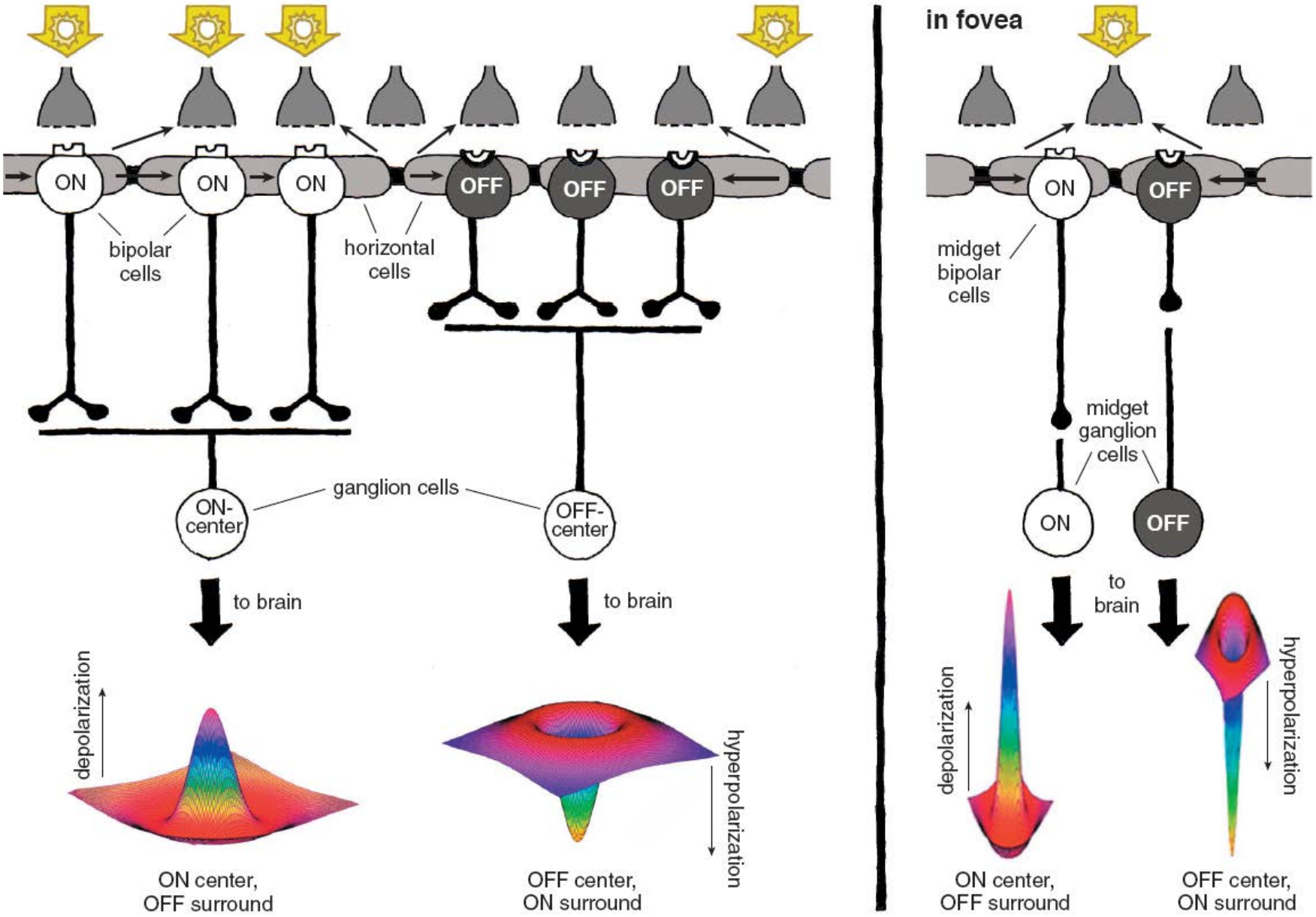




Human retina – regional specialisation



Human retina – regional specialisation



Summary of pathways through the retina

- Photoreceptors always respond to light ON with membrane potential hyperpolarisation, resulting in a reduction of neurotransmitter (Glutamate) release onto Bipolar Cells.

- Bipolar Cells respond to light with either **ON** or **OFF** responses. This is due to the expression of different glutamate receptor types at the photoreceptor-bipolar cell synapse.

- Horizontal cells gather information from many cones and use this to provide an antagonistic, opponent surround element to the bipolar cell receptive field.

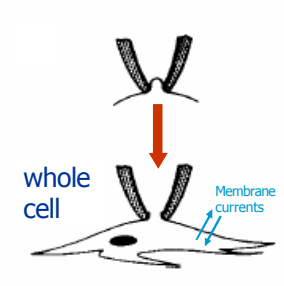
- Bipolar Cells utilise glutamate to synapse onto Retinal Ganglion Cells, conferring them with either **ON** or **OFF** responses.

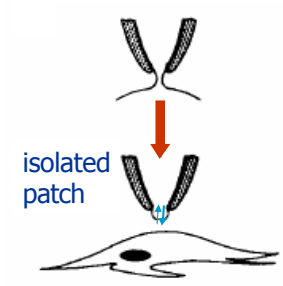
- Retinal Ganglion Cells (RGCs) generate action potentials in responses to graded synaptic input potentials.

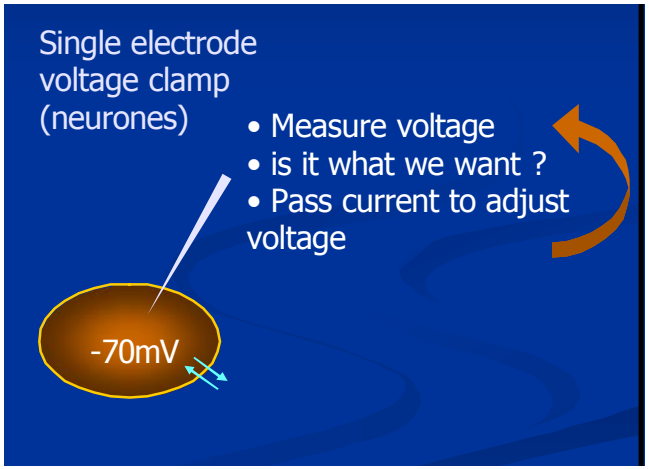
Electrophysiological recording methods

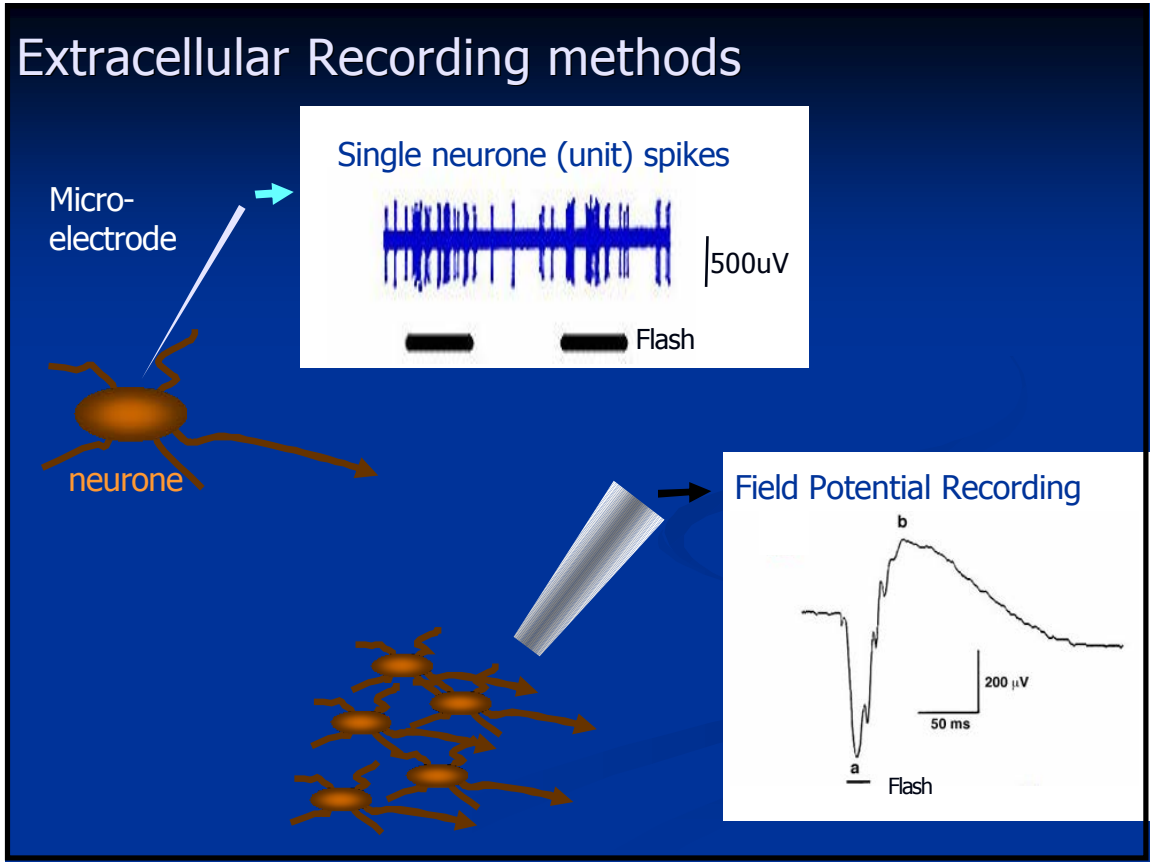
UCL

Whole cell (Patch) recording







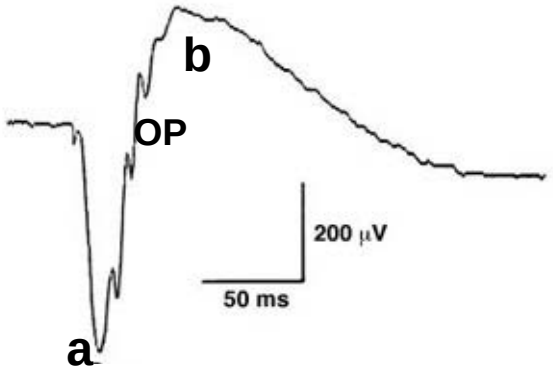


The electroretinogram (ERG) is a widely used field potential recording method

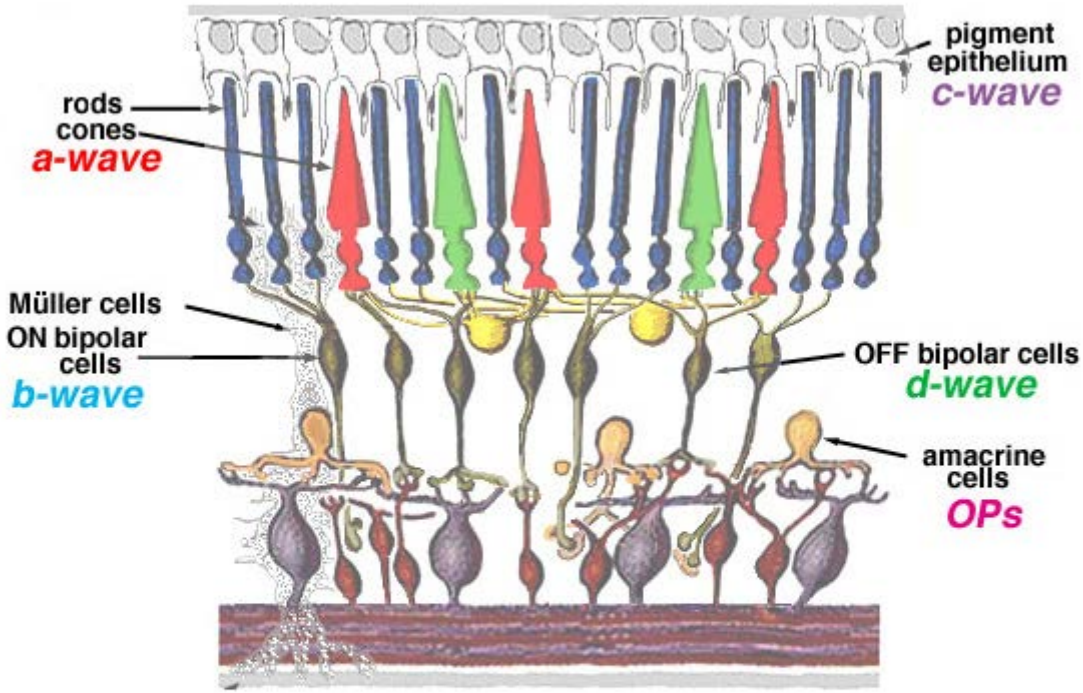
Cellular Origins of the ERG

ERG components

- **a-wave**: photoreceptors
- **b-wave**: ON bipolars (Muller Cells?)
- **c-wave**: pigment epithelium
- **d-wave**: OFF bipolars
- **OP** (oscillatory potentials): amacrine cells



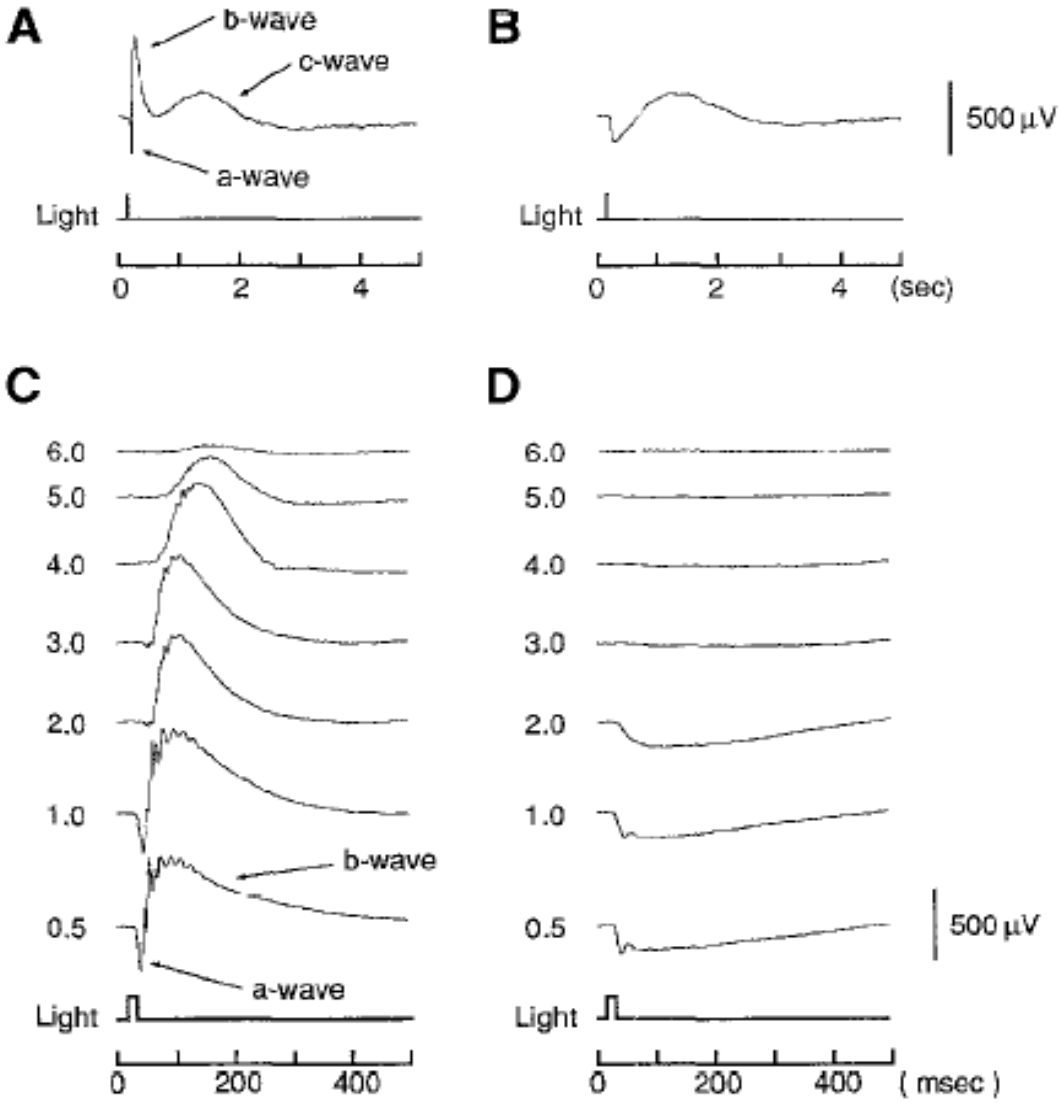
Ragnar Granit, winner of the Nobel Prize for Physiology and Medicine in 1954



ERG in mGlu6 deficient mice

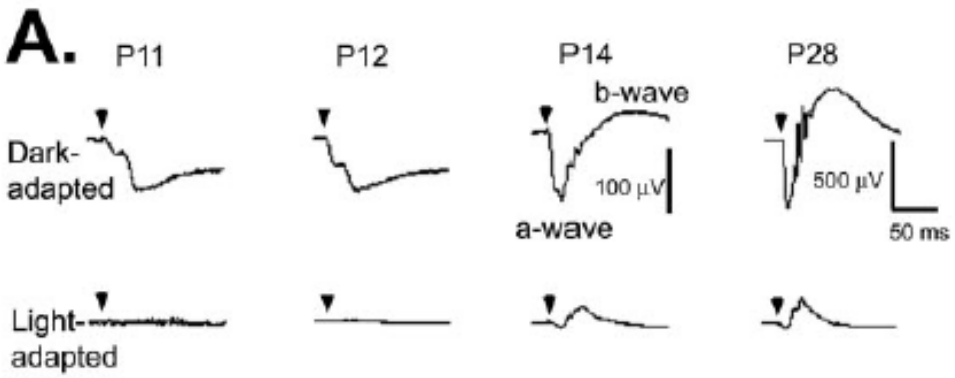
Wild type

mGlu6 deficient



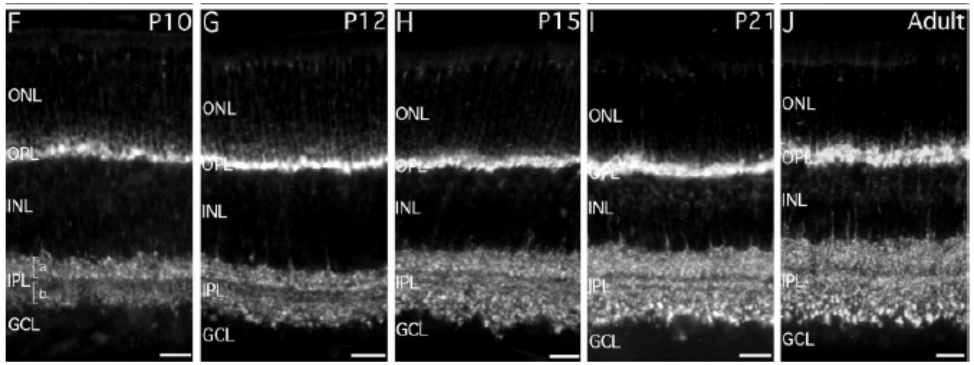
Masu et.al. (1995)
Cell 80[5], 757-765.

The ERG during development



Electroretinogram (ERG) recordings are field potentials measured from the eye's surface (cornea) which reflect the underlying responses of retinal neurons. In mice, the ERG b-wave (an indication of bipolar cell function) develops somewhere between P12 and P14. (Takada et al., IOVS (2004) 45(9): 3302-3312)
Another study using electrodes inserted directly into the retina indicates that rod/cone signalling is not functional until at least P10 in mice. (Tian et al., Neuron (2003) 39: 85-96).

Mice first open their eyelids around P12-P13

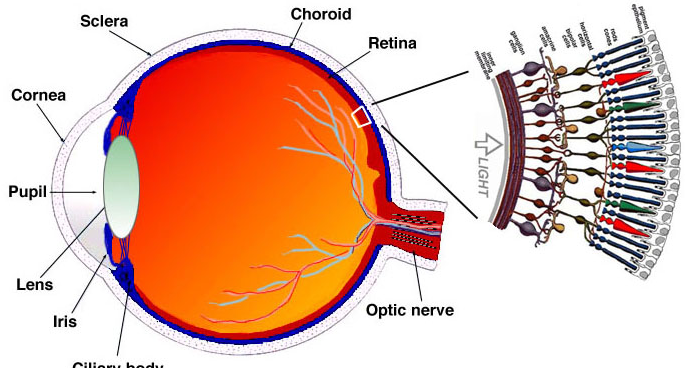


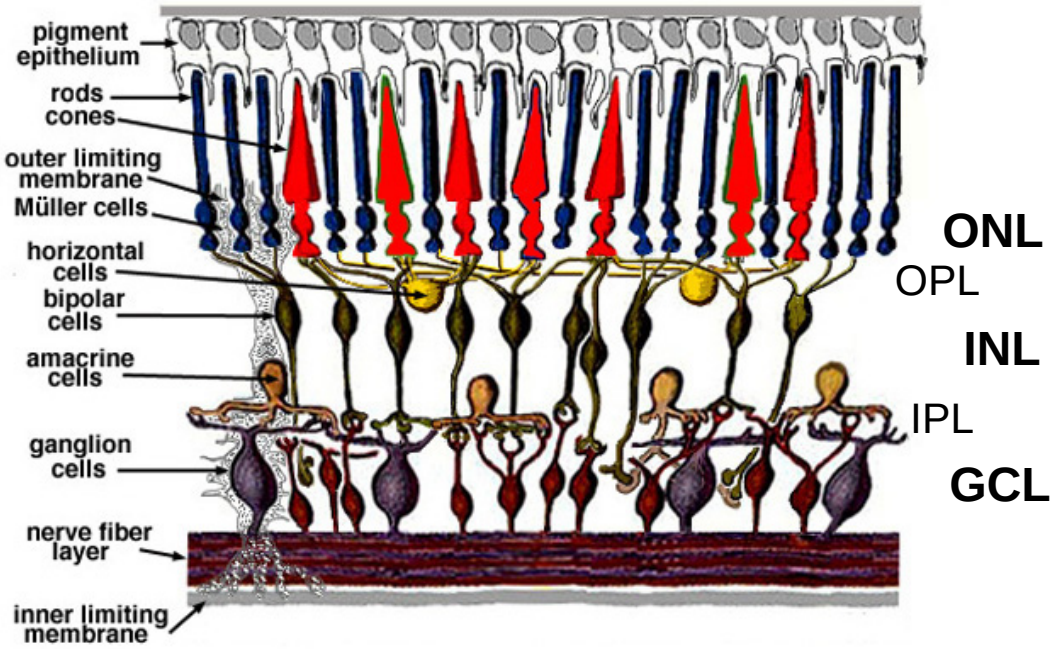
The development of rod, cone and bipolar cell synapses as revealed by immunohistochemistry for vesicular glutamate transporter 1 (VGLUT1). The increase in staining within synaptic layers of the retina (OPL and IPL) corresponds to the enhancement in b-wave between P14 and P28. (Sherry et al., J.Comp Neurol. (2003) 465: 480-498)

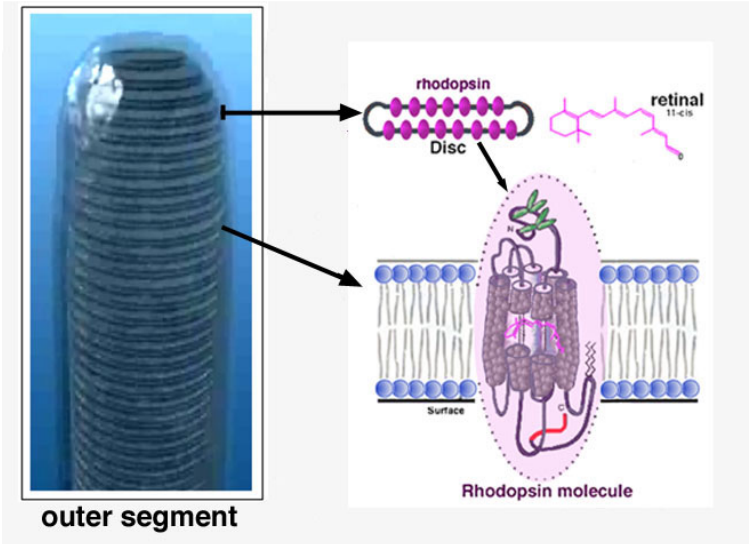
In human neonates, the sensitivity and amplitude of the ERG response is also underdeveloped compared to children of 8 years (Hansen & Fulton IOVS (2005) 46(9) 3458-3462).

Retina – Advanced

Rods and cones account for all photoreceptive input to the mammalian CNS…

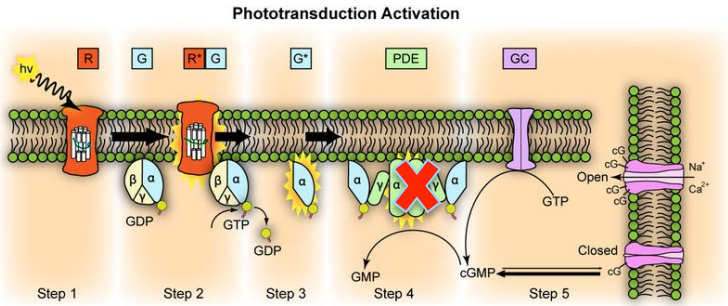


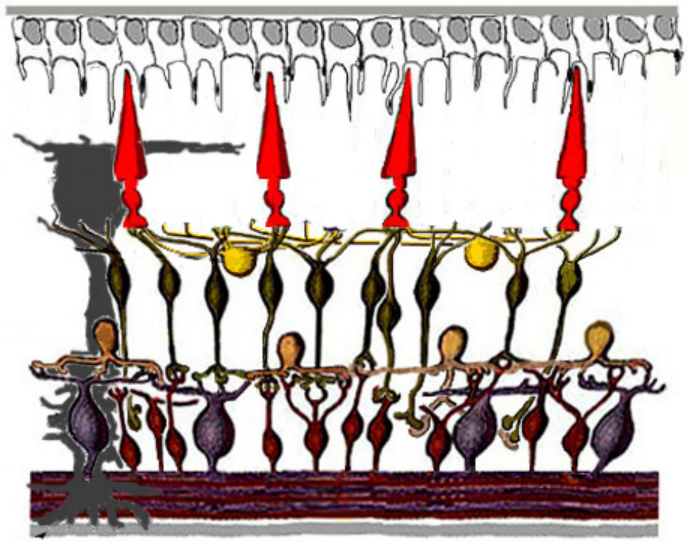




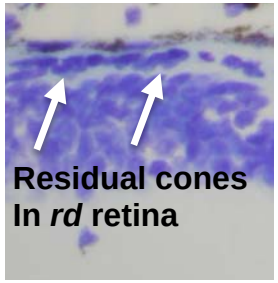
Abbreviations: outer nuclear layer (ONL), outer plexiform layer (IPL), inner nuclear layer (INL), ganglion cell layer (GCL).

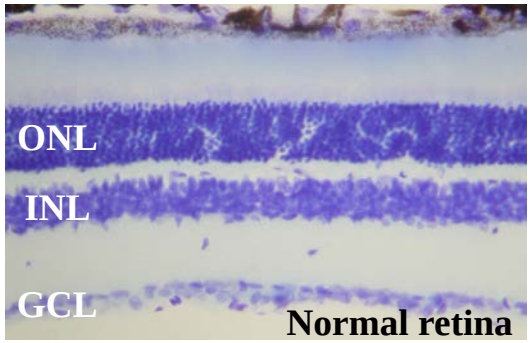
Could there be something else apart from rods and cones?

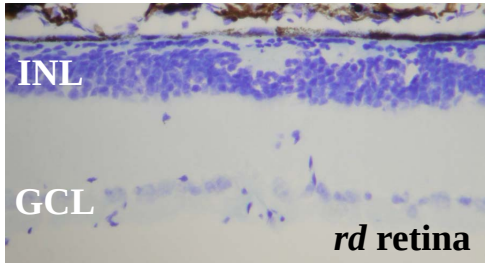




Evidence came from studies of retinal degenerate (*rd*) mice, which have a mutation in the β subunit of rod-specific phosphodiesterase (PDE). This leads to a rapid degeneration of rods followed by a slower loss of cones.







rd mice retain a pupillary light reflex (PLR)

TABLE 1

INDIVIDUAL		EYE	CONDITION OF RETINA	AVERAGE CONTRACTION	TIME OF LATENT PERIOD					TIME OF CONTRACTION					DIAMETER OF PUPIL	
					1	2	3	4	5	1	2	3	4	5	Atropin	Sulfide of eserine
Gray	♀ 28	Left	Normal	1.46–0.616	0.3	0.3	0.3	0.3	0.3	3.0	3.0	3.0	3.0	3.0	2.31	0.231
Black	♂ 23	Left	Normal	1.54–0.539	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.3	3.3	3.3	2.31	0.099
Black	♂ 23	Right	Normal	1.54–0.539	0.6	0.6	0.6	0.6	0.6	3.0	3.3	3.3	3.3	3.3	2.31	0.099
Gray	♀ 12	Left	Normal	1.54–0.539	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.6	4.2	4.2	2.31	0.924
Gray	♀ 12	Right	Normal	1.54–0.616	0.6	0.6	0.6	0.6	0.6	4.2	4.2	4.2	5.6	5.6	2.31	0.924
Gray	♀ 13	Left	Normal	1.54–0.616	0.6	0.6	0.6	0.6	0.6	3.6	3.6	3.6	3.6	3.6	2.31	0.385
Gray	♀ 13	Right	Normal	1.54–0.616	0.6	0.6–	0.6	0.6	0.6	3.6	3.0	3.0	3.0	3.0	2.31	0.385
Gray	♀ 10	Left	Normal	1.54–0.693	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.0	3.0	3.0	2.31	0.154
Gray	♀ 10	Right	Normal	1.54–0.616	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.0	3.0	3.0	2.31	0.154
Chinchilla	♀ 11	Left	Normal	1.54–0.616	0.6+	0.6	0.6	0.6–	0.6	5.4	5.4	5.4	5.4	6.8	2.16	0.616
Chinchilla	♀ 11	Right	Normal	1.54–0.616	0.6+	0.6+	0.6+	0.6+	0.6+	4.8	5.4	5.4	5.4	5.4	2.16	0.616
Averages				1.53–0.602					0.57					3.73	2.28	0.417
Gray	♀ 31	Left	Rodless	1.54–0.62	2.4	2.4	2.7	2.7	2.4	2.4	2.4	2.1	2.1	1.8	2.31	0.154
Gray	♀ 31	Right	Rodless	1.54–0.62	3.0	3.3	2.7	3.0	3.0	2.4	1.5	1.5	2.1	1.8	2.31	0.154
Black	♂	Left	Rodless	2.31–1.16	3.3	3.6				6.0	6.6	Animal choked to death				
Chinchilla	♀	Left	Rodless	1.39–0.61	1.8	1.8	1.8	2.4	1.8	3.0	3.0	3.0	3.0	3.0	2.70	0.385
Chinchilla	♂	Left	Rodless	1.39–1.16	1.2	1.5	1.8	1.8	1.8	3.0	2.4	2.4	2.4	2.4	2.70	0.308
Brown	♂ 7	Left	Rodless	1.93–1.16	1.8	3.0	3.0	3.0	1.8	3.0	2.0	3.0	3.0	2.0	2.39	0.154
Brown	♂ 7	Right	Rodless	1.93–1.16	0.6	0.6	2.4	1.8	2.4	2.4	3.0	1.8	3.0	2.4	2.39	0.154
Brown	♀ 34	Left	Rodless	1.54–0.77	1.8	3.0	3.0	3.0	3.0	2.4	1.8	2.4	3.0	1.8	2.39	0.365
Brown	♀ 34	Right	Rodless	1.54–0.77	1.8	3.0	3.0	3.0	4.8	3.0	1.8	1.8	2.4	1.8	2.31	0.365
Brown	♀ 6	Left	Rodless	1.54–1.16	1.8	1.2	1.8	0.6	0.6	3.0	1.8	2.4	2.4	3.6	2.39	0.231
Brown	♀ 6	Right	Rodless	1.54–1.16	1.8	1.5	1.2	0.6	0.6	2.4	2.4	2.4	3.6	3.6	2.39	0.231
				1.65–9.40					2.18					2.56	2.43	0.250

All diameters are given in millimeters. All times are given in seconds.

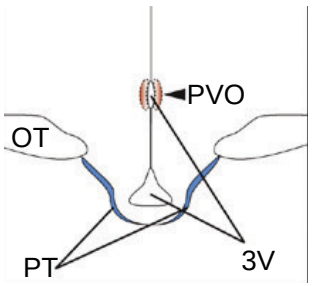
Clyde Keeler noted that rodless animals had a slower and weaker PLR than normals. He concluded that the iris may function independently of vision in rodless animals (based on work in eels from the 1840s) and that the deficits in rodless animals pointed to a regulatory system for iris constriction in normal eyes.

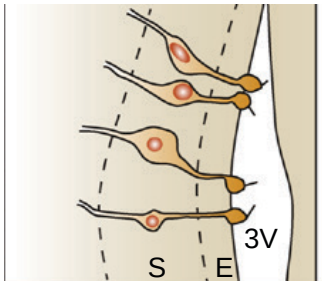
(Keeler, (1927) American J. Physiology 81: 107-112).

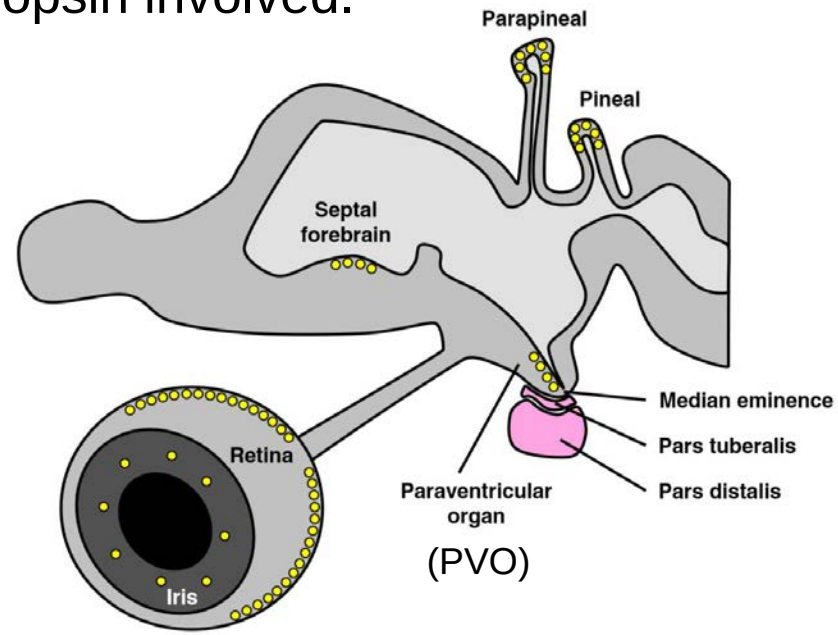
Russell Foster

- Studied the photoperiodic response in quail
  - PhD in the Dept. Zoology University of Bristol
- Seasonal gonad maturation is mediated by deep brain photoreceptors in the hypothalamus.
  - Foster et al., (1985) Nature 313(3): 50-52.
  - Defined the action spectrum of the opsin involved.

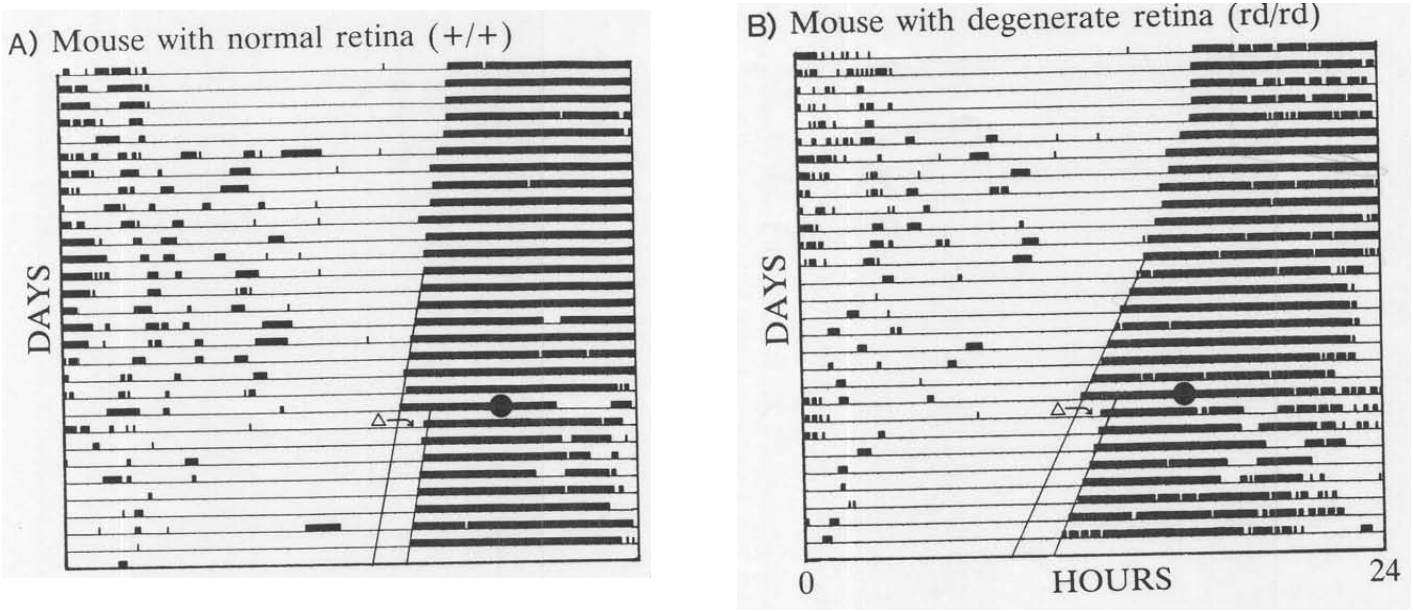
Birds have deep brain photoreceptors in the hypothalamus







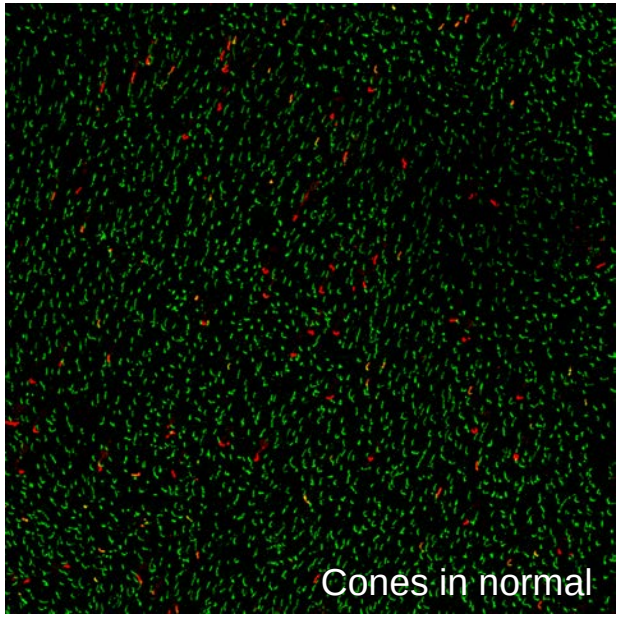
rd mice retain circadian photoreception

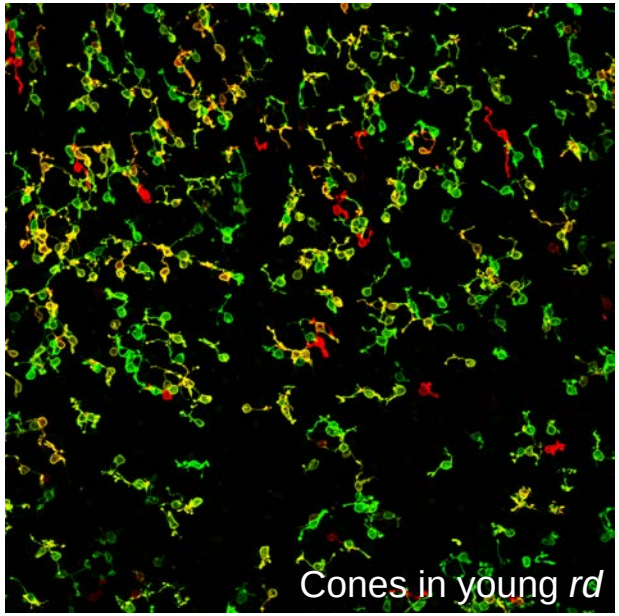


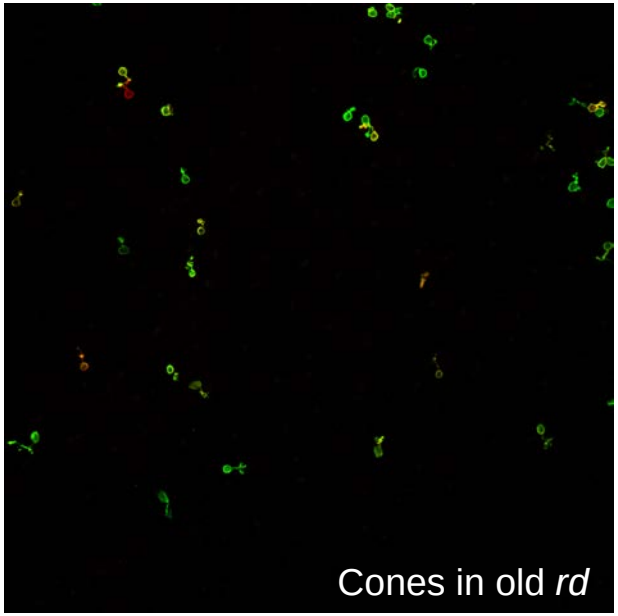
- Mice on 12h light:dark cycle for the first 5 days, then into constant darkness for 16 days. Black bars show wheel running activity during subjective night. A 15min pulse of light at CT16 (●) causes a 90min delay in the phase shift (Δ) on subsequent days in both normal and *rd* mice. Testing at different irradiances revealed that the response in *rd* mice was indistinguishable from that in congenic wildtype (+/+) mice. This was in contrast to an earlier study by Ebihara and Tsuji in 1980, which compared *rd* mice with wildtype mice from a different strain.

(Foster et al., (1991) J. Comp Physiol A 169: 39-50).

Could cones mediate this circadian response in *rd* mice?



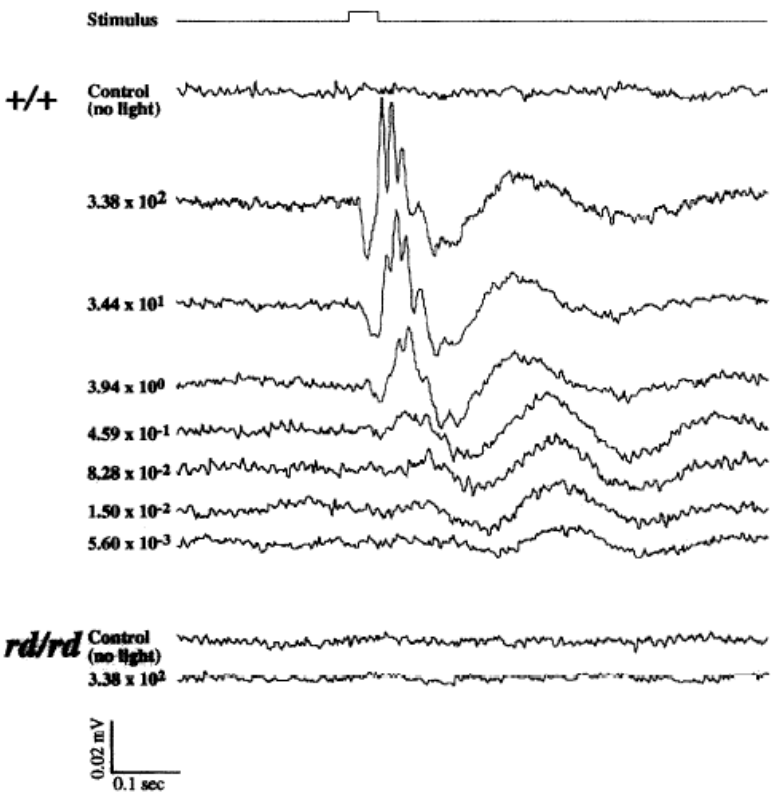




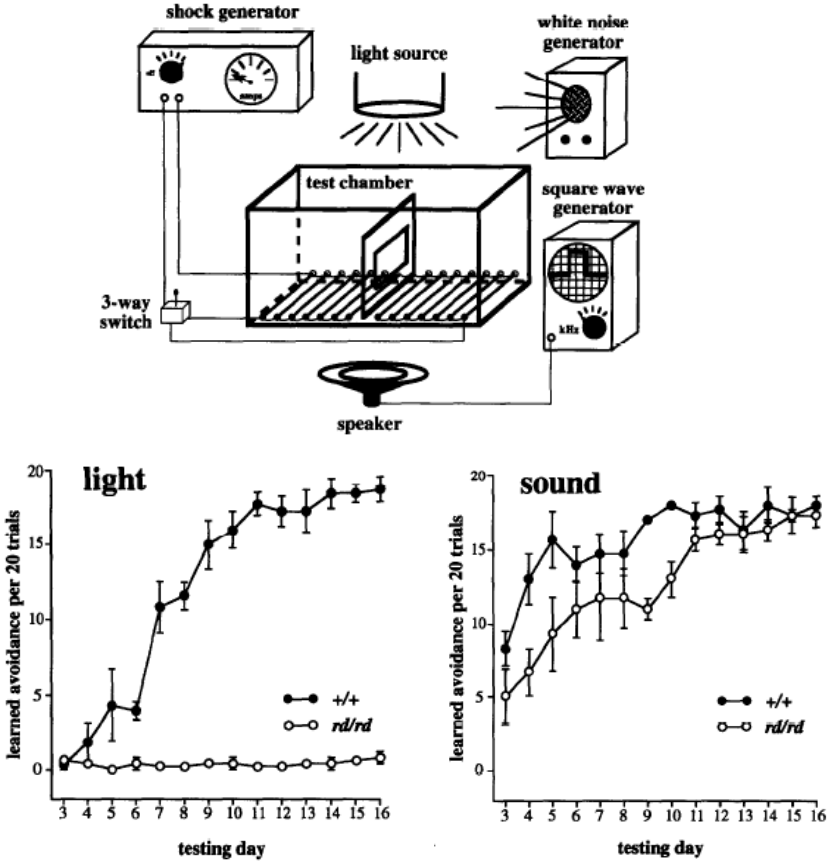
Images above show immunohistochemistry (antibody staining) for Short-wavelength sensitive cone opsin (red) and Long-wavelength sensitive cone opsin (green). The *rd* mouse lacks rods but retains cones, which decline in number with age.

Ignacio Provencio and Russell Foster went on to show that even in old (>2 years) *rd* mice, the ability to phase shift in response to light remained indistinguishable from age matched normal mice (Provencio et al., (1994) Vision Res. 34(14) 1799-1806).

Old *rd* mice appear to be otherwise blind



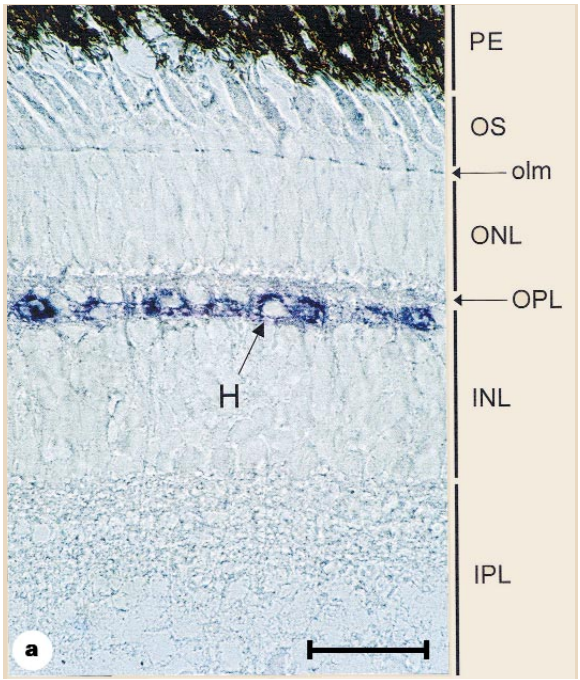
rd mice have no detectable ERG from 26 days old.

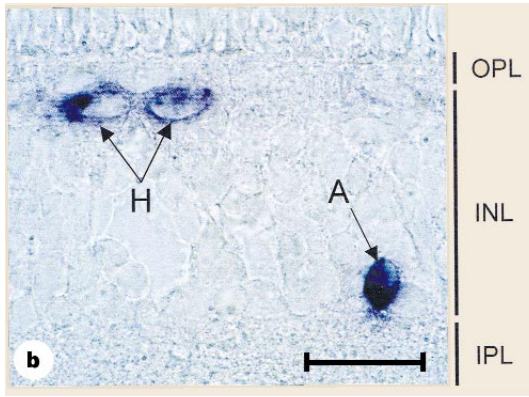


The *rd* mice failed to associate light with impending shock.

(Provencio et al., (1994) Vision Res. 34(14) 1799-1806)

Russell Foster was convinced there was another opsin at work in the vertebrate retina apart from rhodopsin and cone opsins…

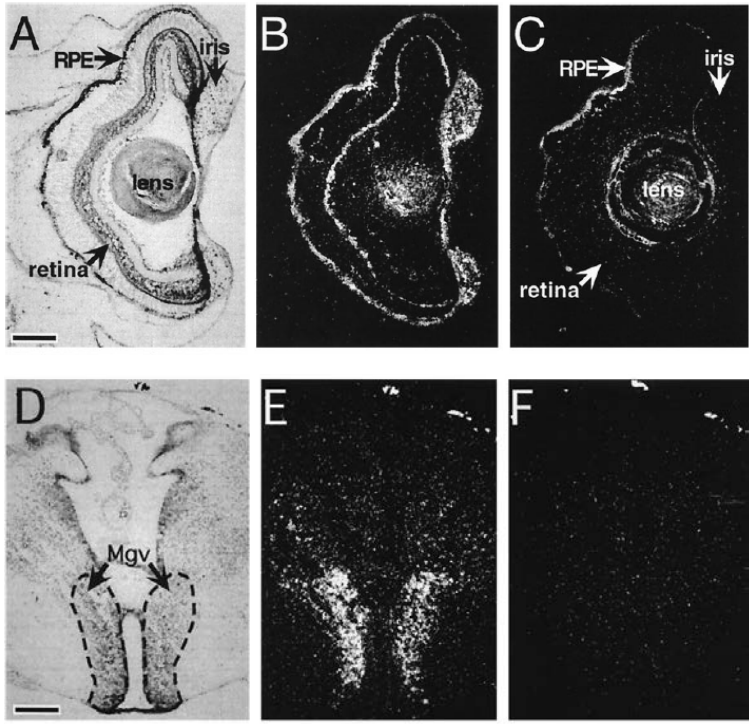




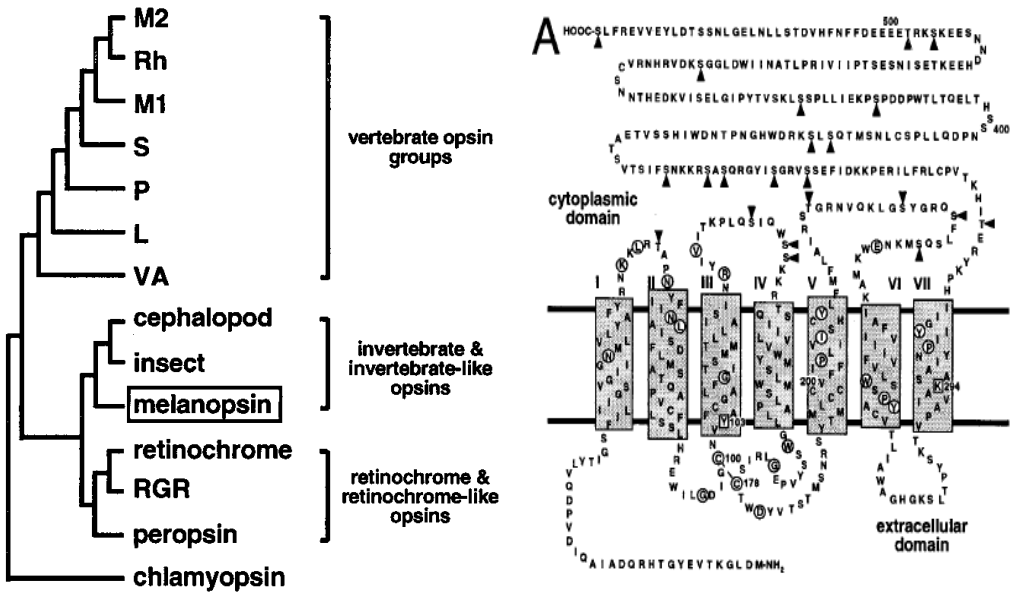
(Soni, Philip & Foster (1998) Nature 394: 27-28)

In situ hybridisation histochemistry (ISHH) revealed that vertebrate ancient (VA) opsin is expressed in horizontal (H) and amacrine (A) cells of the fish retina

Ignacio Provencio discovers melanopsin in photosensitive dermal melanophores, brain and eye of *Xenopus laevis*



ISHH for melanopsin mRNA shows antisense signal (white) in the retina (INL) / iris (B) and hypothalamus (E). The adjacent sections (C&F) are sense probe controls.

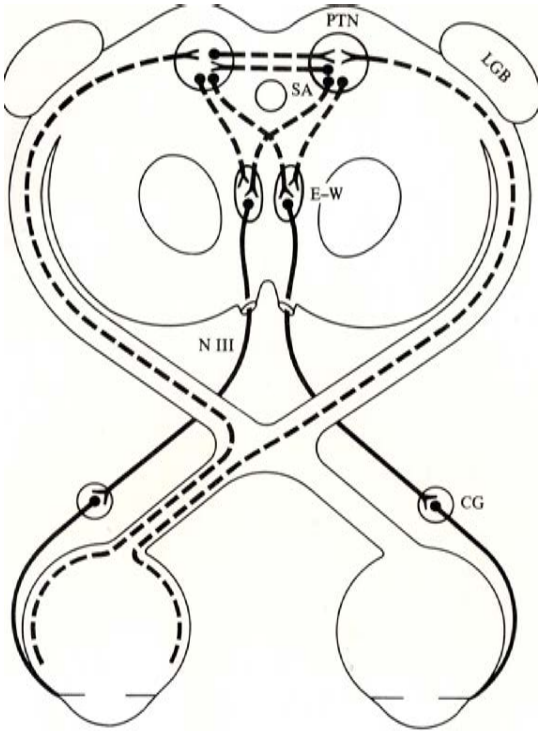


(Provencio et al., (1998) *Proc. Natl. Acad. Sci. USA* 95(6) 340-345)

Foster lab at Imperial College London generated mice lacking rods and cones (*rd/rd cl* mice)

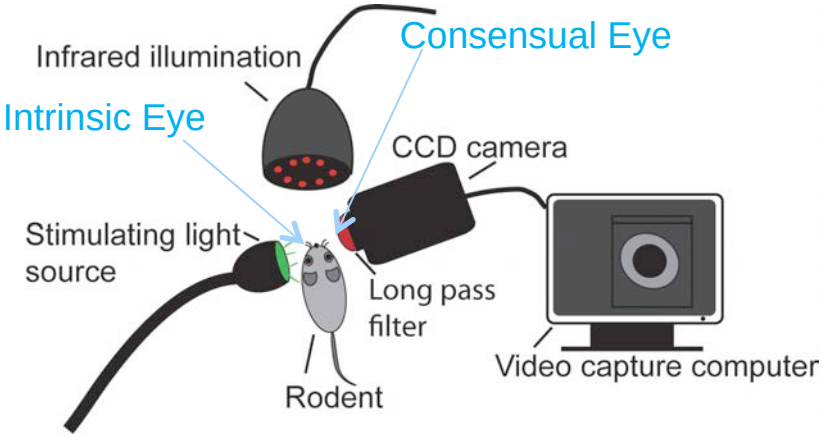
- Following a 15 minute exposure to green light the *rd/rd cl* mice still had:
  - Circadian phase shifting (Freedman et al., **Science** (1999) 284 502-504)
  - Suppression of pineal melatonin (Lucas et al., **Science** (1999) 284 505-507)
- The *rd/rd cl* mice also retain a pupillary light reflex (PLR)
  - Lucas, Douglas and Foster (2001) **Nature Neuroscience** 4(6) 621-626

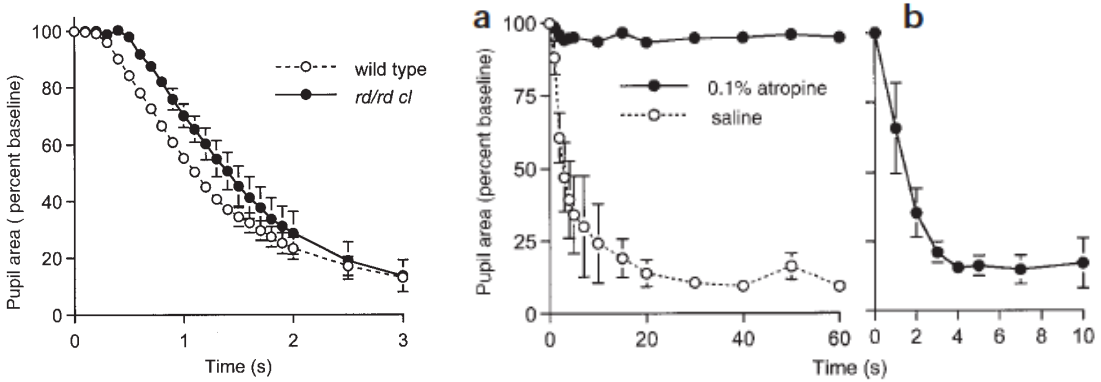
A non-rod, non-cone photoreceptor regulates the Pupillary Light Reflex (PLR)



Neural circuitry of the PLR

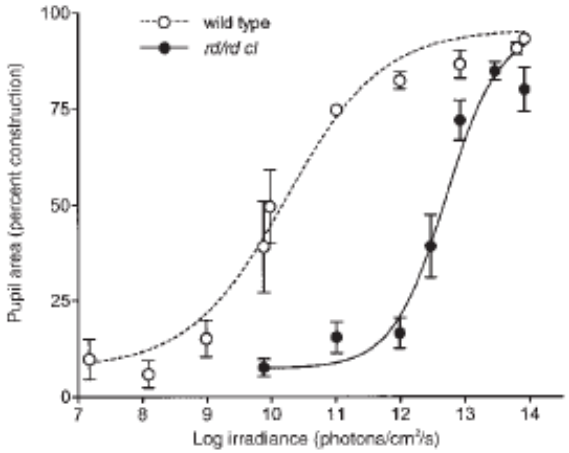
(Lucas et al., (2001) Nature Neuro. 4(6) 621-626)



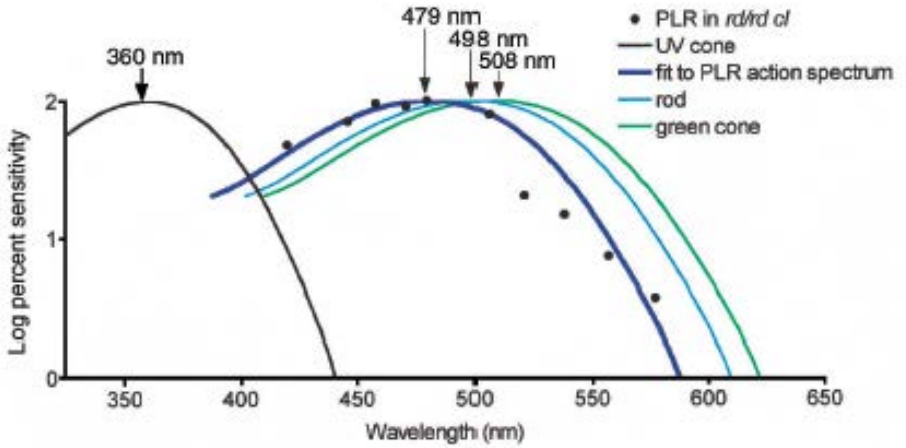


The PLR in *rd/rd cl* mice is abolished by topical atropine application (a) and can be elicited in the consensual eye (b). Therefore, the response is mediated by connections to the brain (3 mW/cm^2 white light).

The spectral properties of this new photoreceptor were defined using the PLR in *rd/rd cl* mice



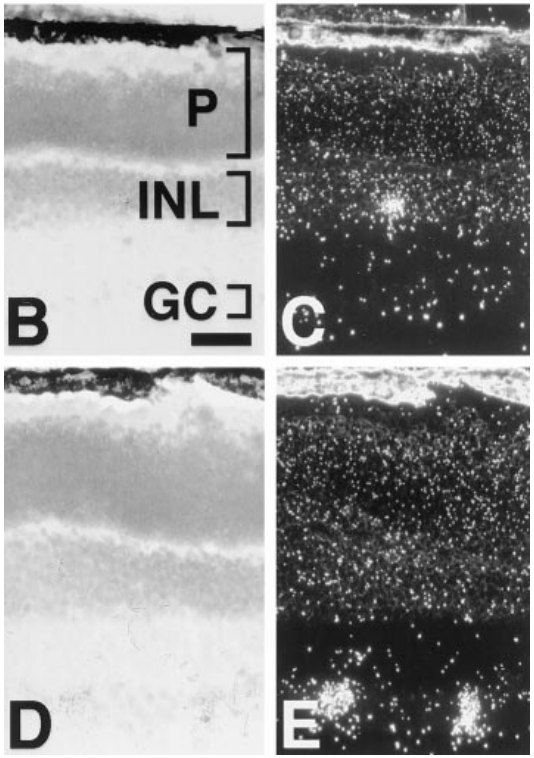
Irradiance response to
506 nm monochromatic light



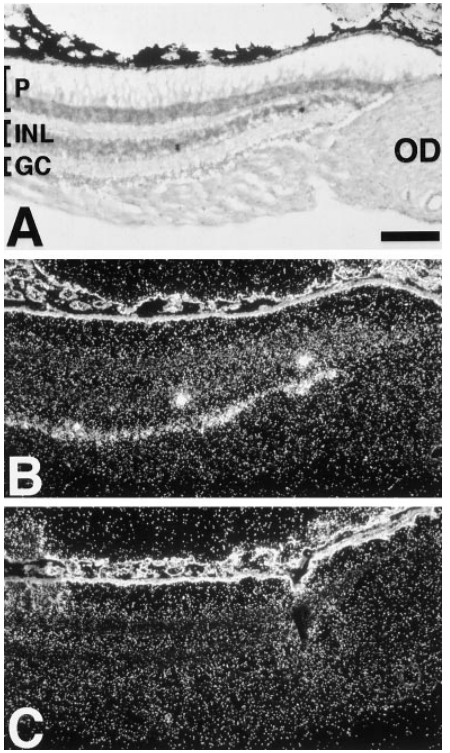
The action spectrum for the unidentified Photopigment peaks at 479nm (OP^{479})

(Lucas et al., (2001) Nature Neuro. 4(6) 621-626)

ISHH reveals melanopsin in the inner retina of mammals



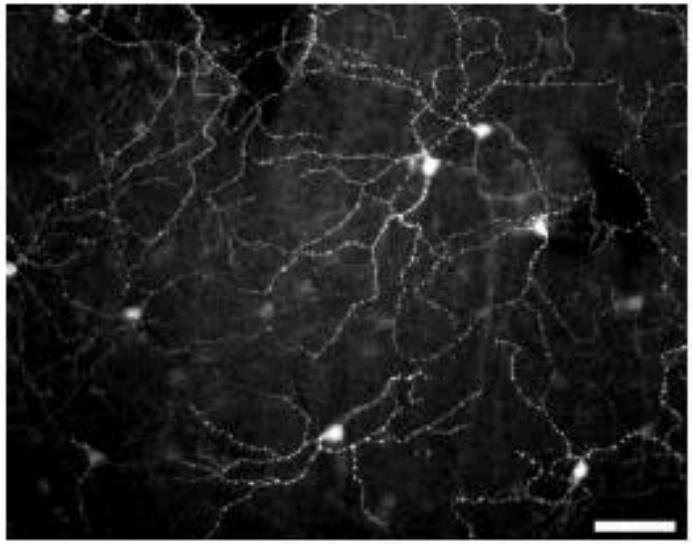
Mouse



Monkey

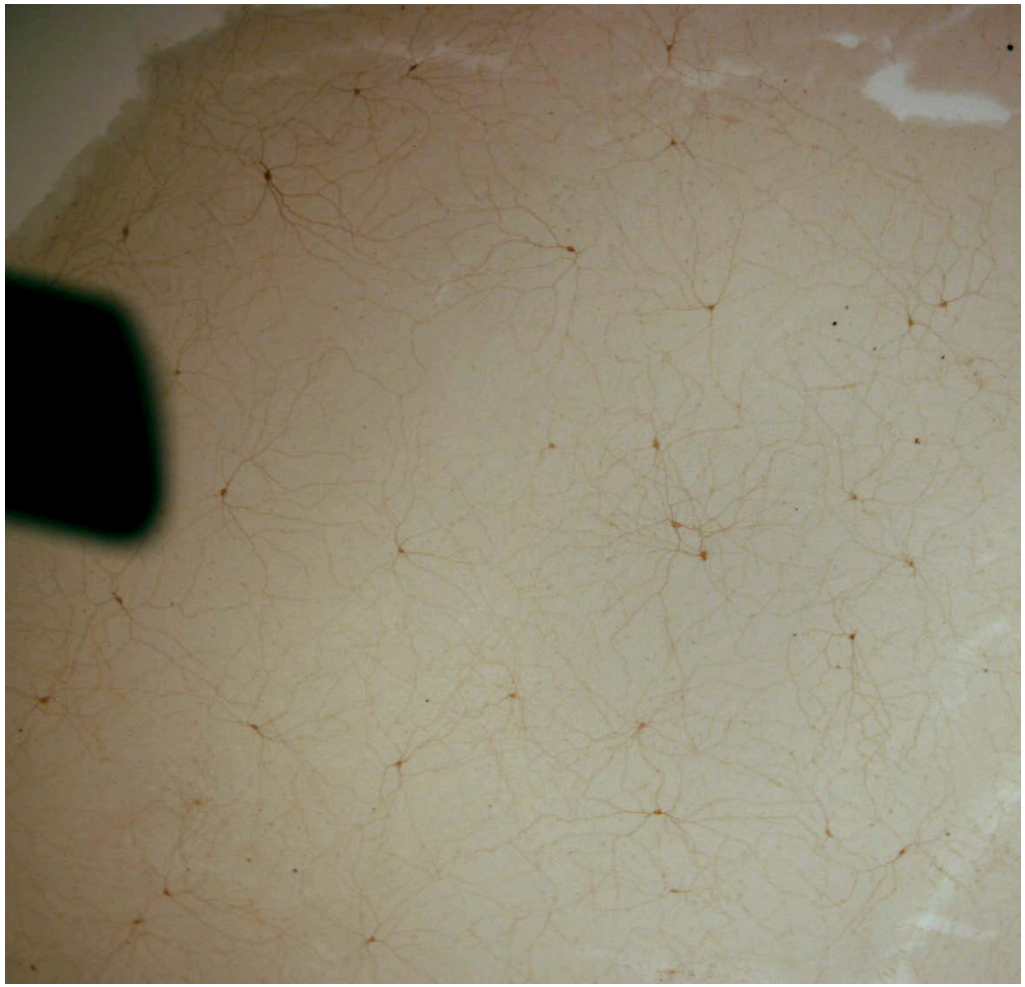
(Provencio et al., (2000) J. Neurosci. 20(2) 600-5)

Antibodies to melanopsin reveal a network of ganglion cells in the inner retina

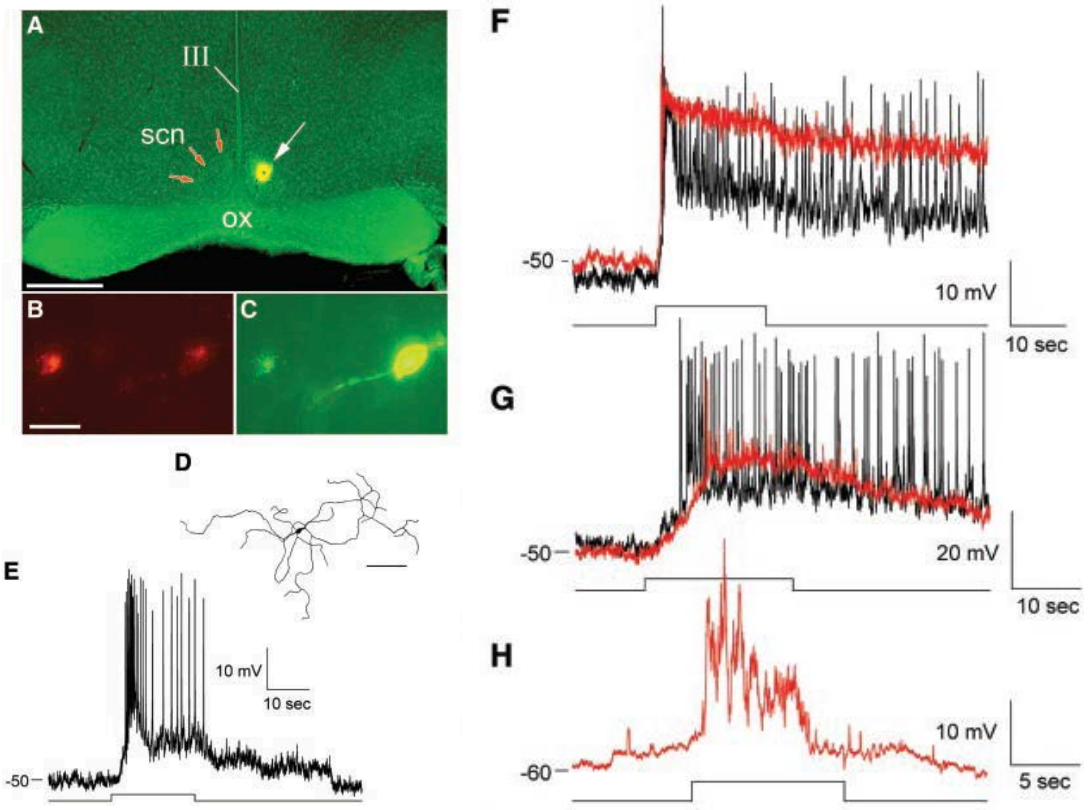


Above: Anti-melanopsin antibody revealed a network of cells in the inner retina of mice (Provencio et al., Nature (2002) 415 493).

Right: Melanopsin cells in the human retina



Ganglion cells of the retinohypothalamic tract shown to be intrinsically photosensitive and referred to as intrinsically photosensitive RGCs



Berson et al., Science (2002) 295 1070-73

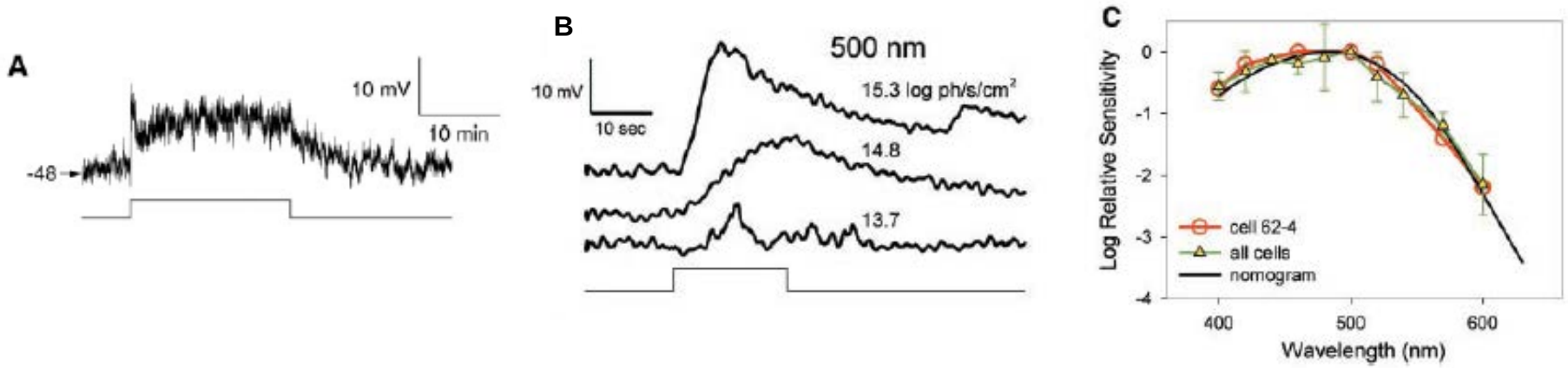
It had been suspected that retinal ganglion cells which project to a specialised region of the hypothalamus called the suprachiasmatic nucleus (SCN) may be the melanopsin positive cells.

Fluorescent beads were injected into the rat SCN. This retrogradely labeled ganglion cells in the retina which were found to depolarise in response to prolonged light exposure (measured using whole-cell patch clamp recordings).

The intrinsic light response was demonstrated by bathing the cells in 2mM $CoCl_2$ (red traces), either alone (F), or together with additional drugs to block glutamatergic signals from rods/cones (G).

Physically isolated cells (H) also retained Their intrinsic light response.

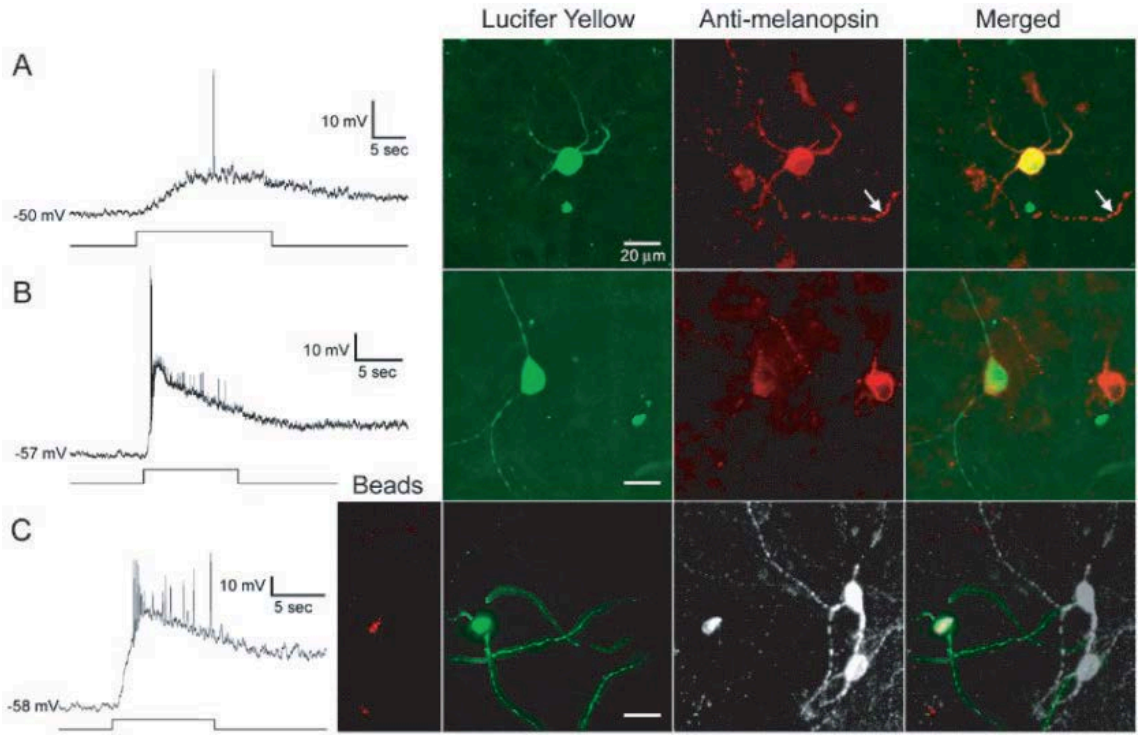
Properties of the intrinsic light response are similar to the irradiance response properties of the PLR



The electrophysiological response of intrinsically photosensitive retinal ganglion cells can be sustained for long periods of time (**A**) and is dependent on irradiance (**B**), with a peak sensitivity around **480nm** (**C**).

Berson et al., Science (2002) 295 1070-73

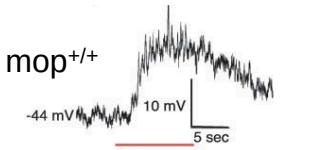
These cells also express melanopsin

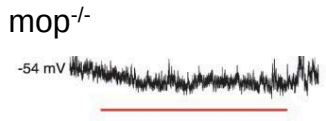


Retinal ganglion cells were retrogradely labeled (from the SCN) with fluorescent beads to enable whole-cell recordings. At the end of recording, cells were filled with the fluorescent dye Lucifer yellow (green). Labeling of these cells with an antibody against rat melanopsin confirmed their identity.

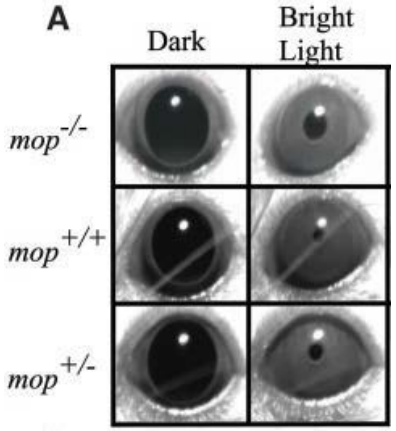
Hattar et al., Science (2002) 295 1065-70

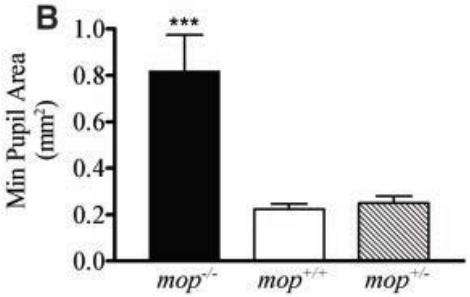
Melanopsin-knockout eliminates the intrinsic light response of ipRGCs and reduces the PLR at high Irradiance

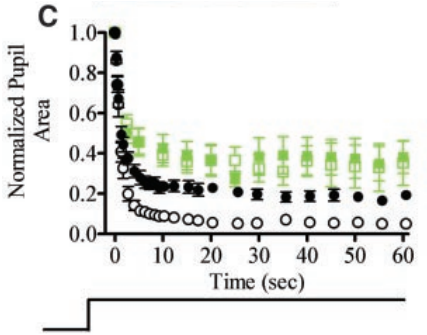




Electrophysiology







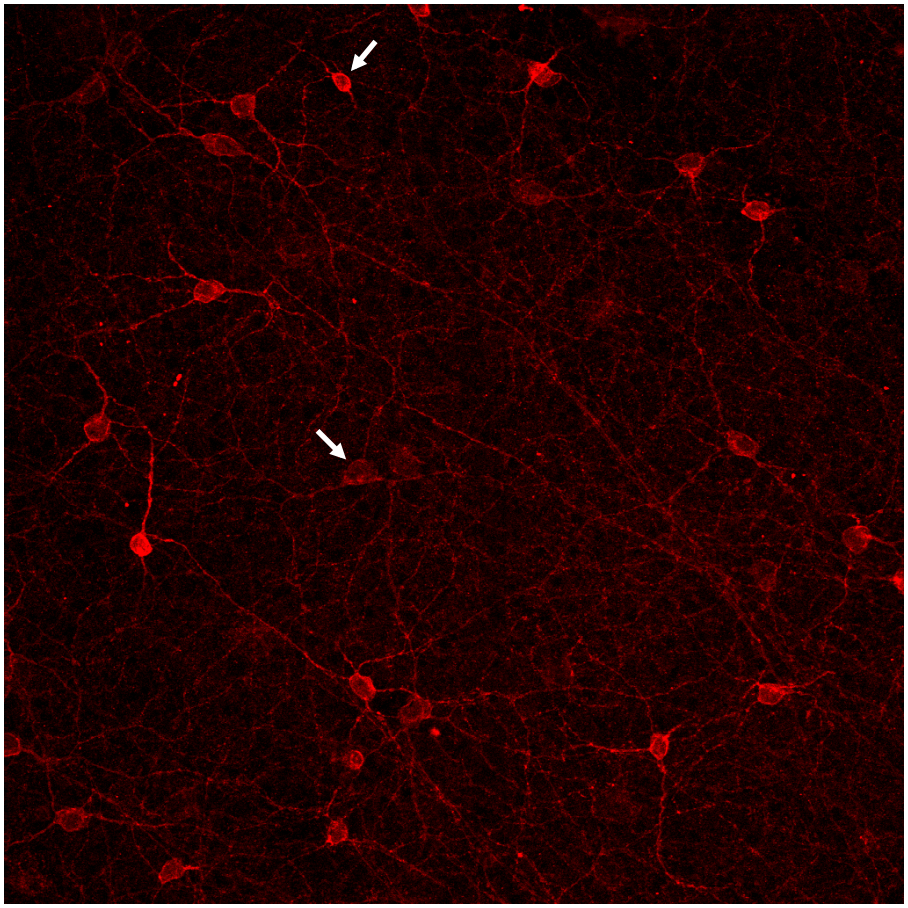
Melanopsin-knockout (*mop*$^{-/-}$) mice were generated, where the ipRGCs remain but lack melanopsin and do not respond intrinsically to light (see intrinsic light responses on the left). As shown in **A** and **B**, unlike wildtype (*mop*$^{+/+}$) and heterozygote (*mop*$^{+/-}$) mice, *mop*$^{-/-}$ mice could not quite achieve a full pupil constriction under bright light (monochromatic 480nm, 145µW cm^2). The *mop*$^{-/-}$ mice can sustain pupillary constriction for 60 seconds like wildtypes (**C**) and can sustain the same level of constriction under low irradiance (0.12µW cm^2, green squares) but not high irradiance (110 µW cm^2, black circles).

Lucas et al., Science (2003) 299 245-247

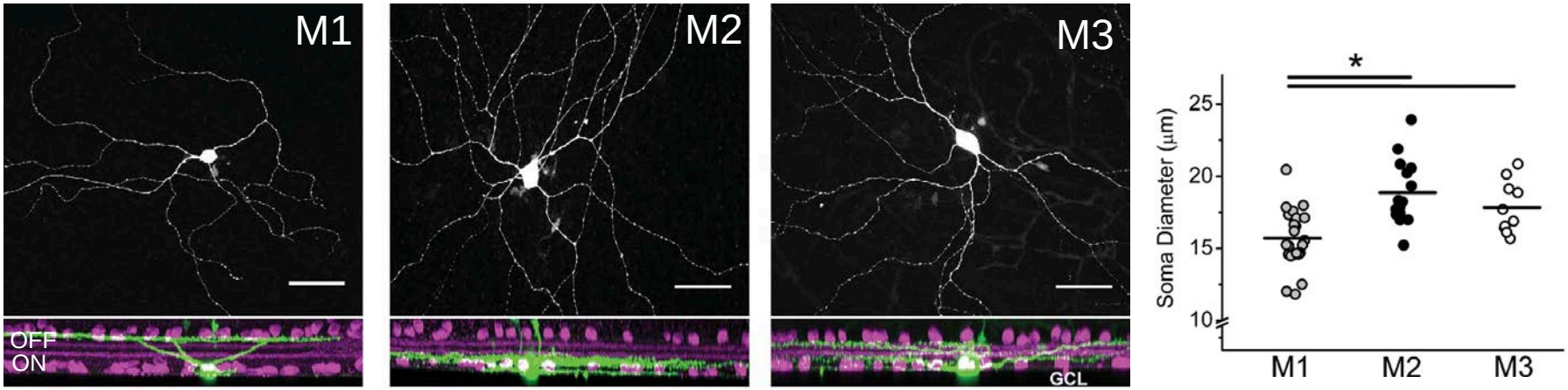
Overview:

- The discovery of intrinsically photosensitive Retinal Ganglion Cells (ipRGCs)
- Structure and function of ipRGCs
  - Anatomy and physiology
  - How do ipRGCs contribute to visual function?

There is more than one type of ipRGC in the mouse retina



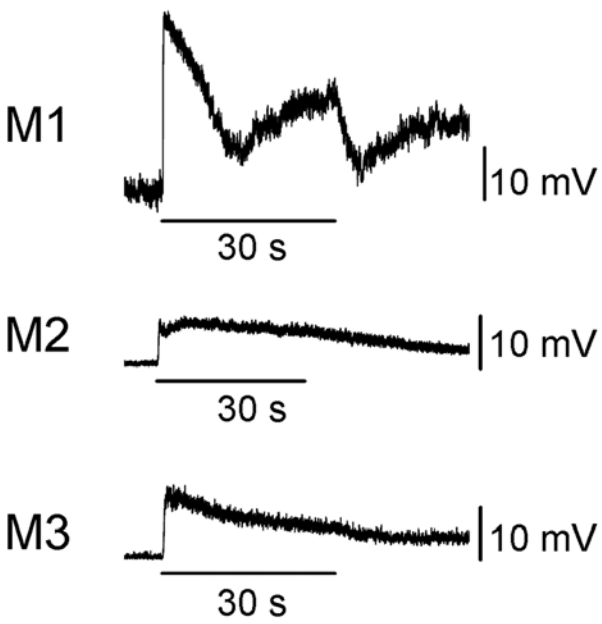
Three main types of ipRGC were originally distinguished on the basis of dendritic stratification

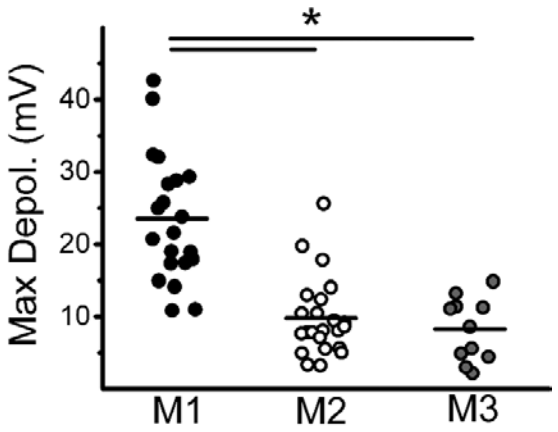


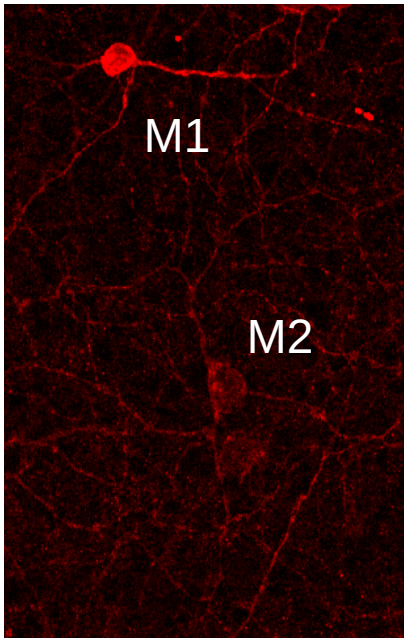
The three types of ipRGC (M1, M2 and M3) are shown in green (filled with neurobiotin), with a marker for cholinergic amacrine cells in magenta (to delineate ON and OFF sub-regions of the inner plexiform layer). The M1 cells (smallest soma diameter) extend dendrites into the OFF subdivision, while M2 cells extend dendrites into the ON subdivision only. M3 cells extend dendrites into both ON and OFF regions (Schmidt and Kofuji J. Comp Neurol. (2011) 5(19) 1492-1504).

In general, M1-type ipRGCs have smaller cell bodies and express higher levels of melanopsin.

The different types of ipRGC have distinct electrophysiological responses to light



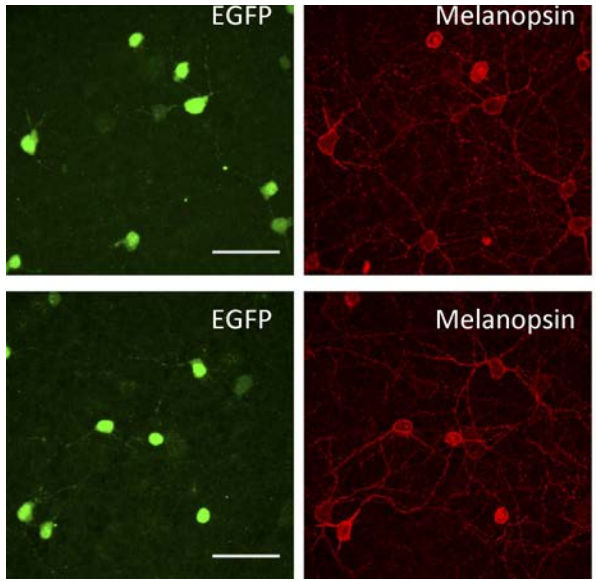


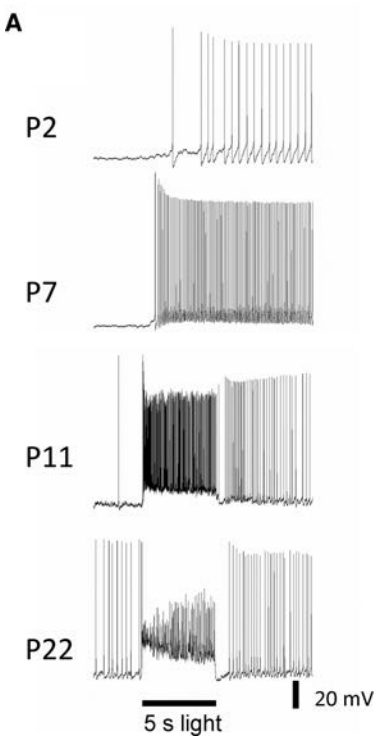


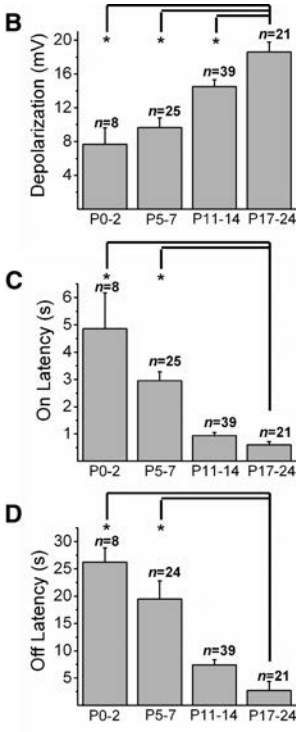
Patch-clamp recordings from ipRGCs in Opn4-EGFP mice (in the presence of synaptic blockade), reveal a stronger depolarisation to bright white light in M1-type cells. This is because M1 ipRGCs contain the highest levels of melanopsin (Opn4, stained red).

Schmidt and KofujiJ. Comp Neurol. (2011) 5(19) 1492-1504

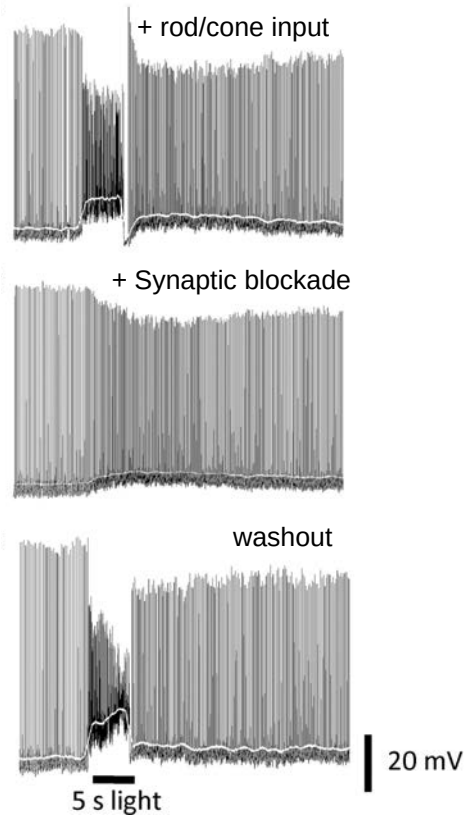
Outer retinal input modulates the ipRGC light response







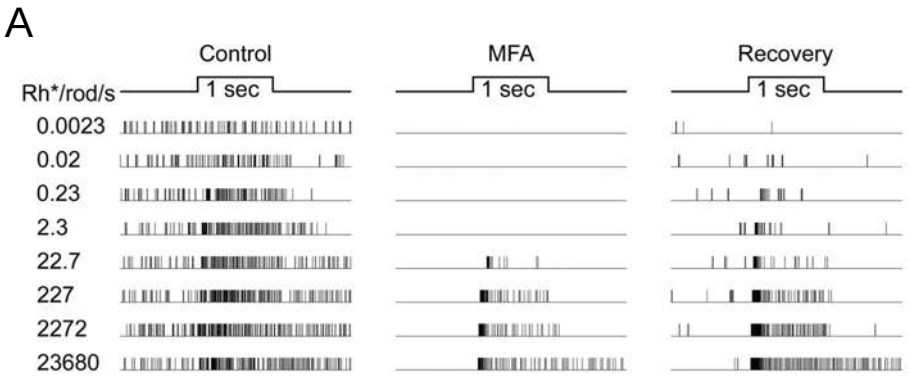
P21 ipRGC response



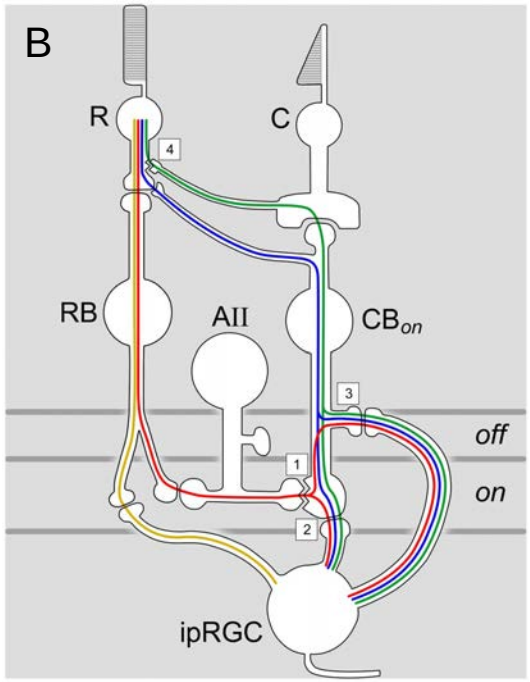
Schmidt et al., *J. Neurophysiol.* (2008) 100: 371 - 384

Whole cell patch-clamp recordings were made from ipRGCs in mice where enhanced green fluorescent protein (EGFP) is expressed under control of the melanopsin promoter. Strong evidence of rod/cone input from P11, with increased amplitude of ipRGC depolarisation and reduced On and Off latencies. A similar response is seen in postnatal day 21 (P21) mice plus or minus synaptic blockade induced by a cocktail of drugs that block glutamate receptors (right panel).

ipRGCs receive sustained ON-type input from rods and cones



Weng et al., PLoS One. (2013) 8(6), e66480



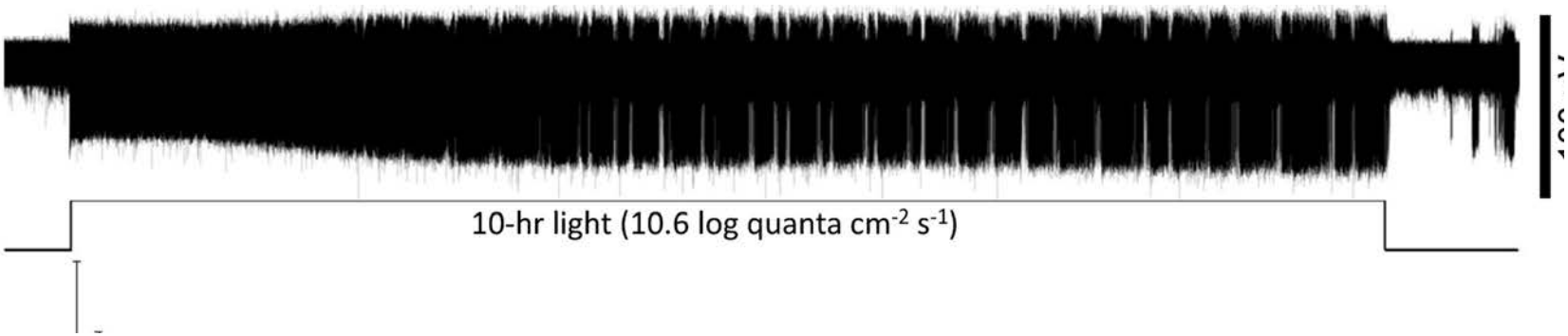
Rod (R)
Cone (C)
Rod bipolar (RB)
ON cone bipolar (CBon)
AII amacrine (AII)

IPL

The ipRGC illustrated here is a hybrid of known types

A. Pharmacological blockade of gap junctions with 100µM meclofenamic acid (MFA) reduces sensitivity of synaptic input to ipRGCs. Light intensity is in Rh*/rod/S (average rate of photoisomerisations/rod/second) and each line represents responses from a single ipRGC exposed to light stimuli (500nm) of increasing intensity (~1log unit increase per line). **B**. Schematic summary of routes by which rod signals could reach ipRGCs. Jagged lines indicate gap junctions at sites 1 and 4. The primary rod pathway is in red, secondary rod pathway in green and other novel pathways in blue and gold. It has been shown that M1 type ipRGCs predominantly receive "ON" type input despite ramifying in the "OFF" sublamina of IPL. This is due to "***en passant***" synapses made by ON bipolar cells as they pass through the OFF sublamina of IPL (Dumitrescu et al., J. Comp. Neurol. (2009) 517, 226-244; Hoshi et al., J. Neurosci. (2009) 29, 8875-83).

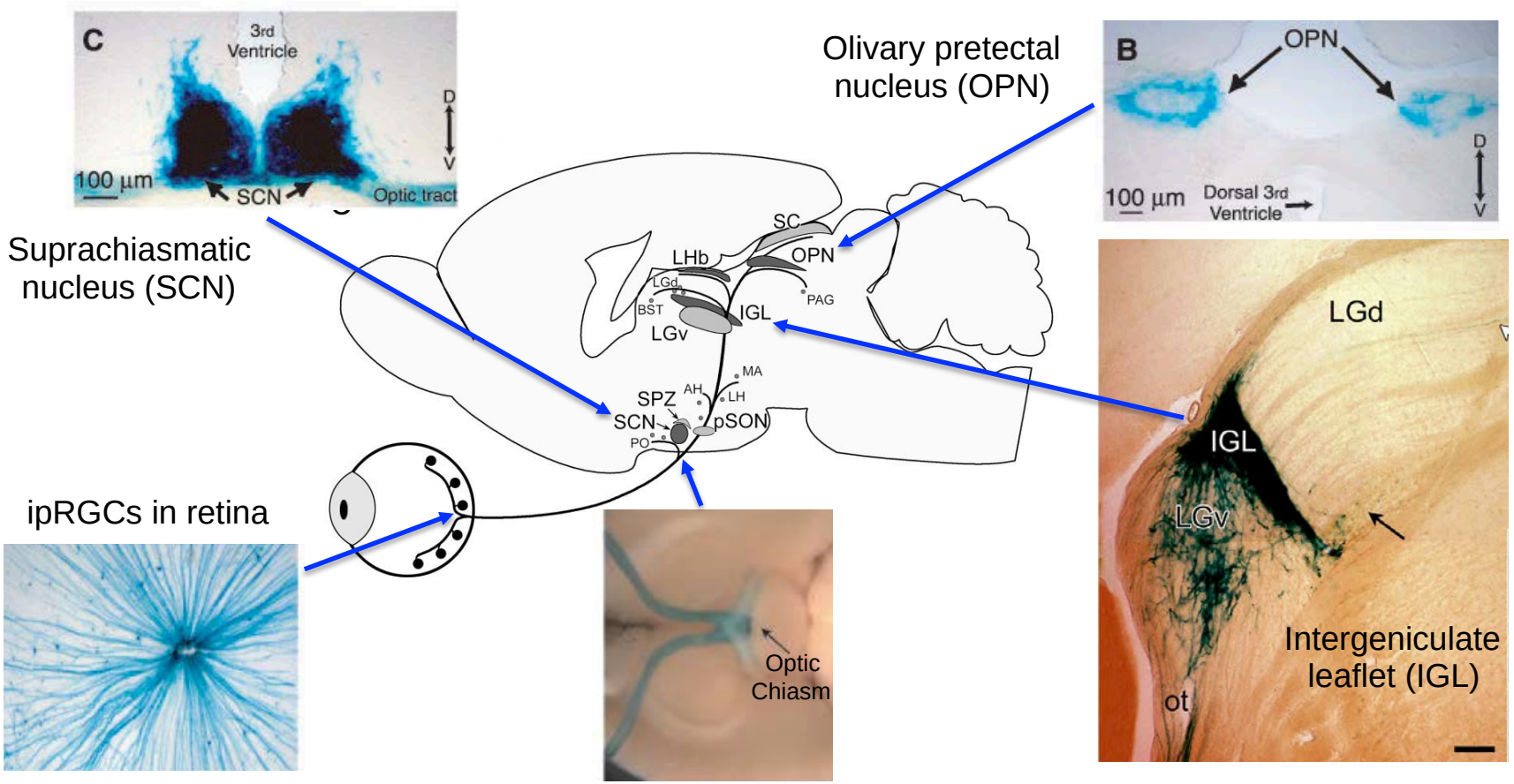
ipRGCs signal irradiance throughout the day



Wong, *J. Neurosci.* (2012) 32(33): 11478 - 11485

The recording above is from a single ipRGC in the absence of synaptic blockade. Similar recordings from ipRGCs in *Opn4*$^{-/-}$ mice also revealed tonic firing in ipRGCs driven by rods/cones.

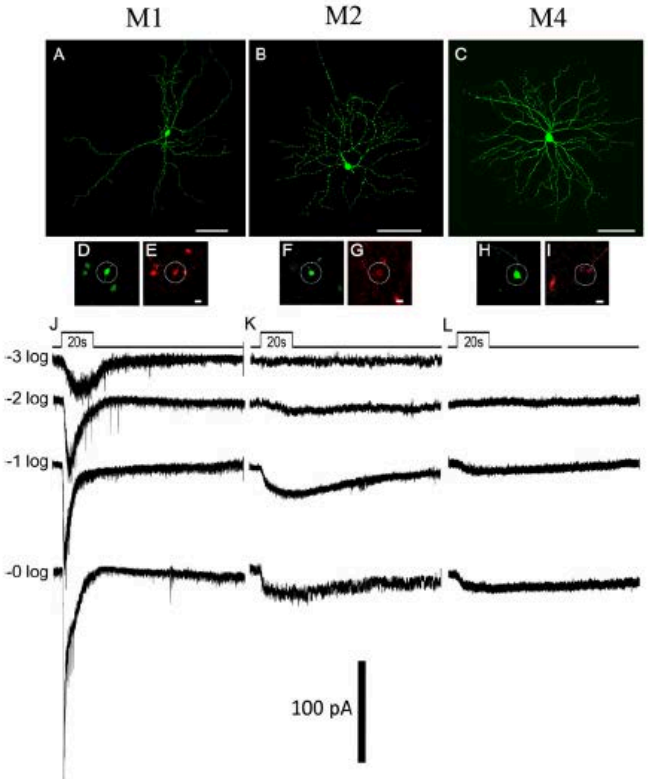
Where do ipRGC axons project to in the brain?

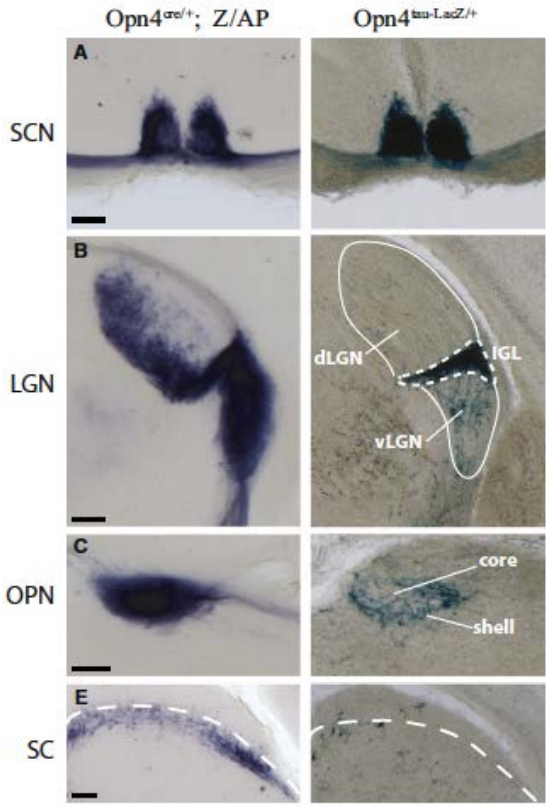


The sub-cortical targets of ipRGCs were first identified using melanopsin-knockout (*Opn4*$^{-/-}$) mice, where the melanopsin gene (*Opn4*) is replaced by a gene for *tau-LacZ*. Axons can be visualised in these mice because the β-galactosidase enzyme is transported along axons due to the inclusion of *tau*. ipRGC axons are stained blue using the enzyme substrate (X-Gal staining). The main brain targets are shown in dark gray in the central diagram (sagittal plane).

Hattar et al., J. Comp Neurol. (2006) 497 326-49

A different reporter mouse reveals new types of ipRGC and more extensive projections to the dLGN and SC



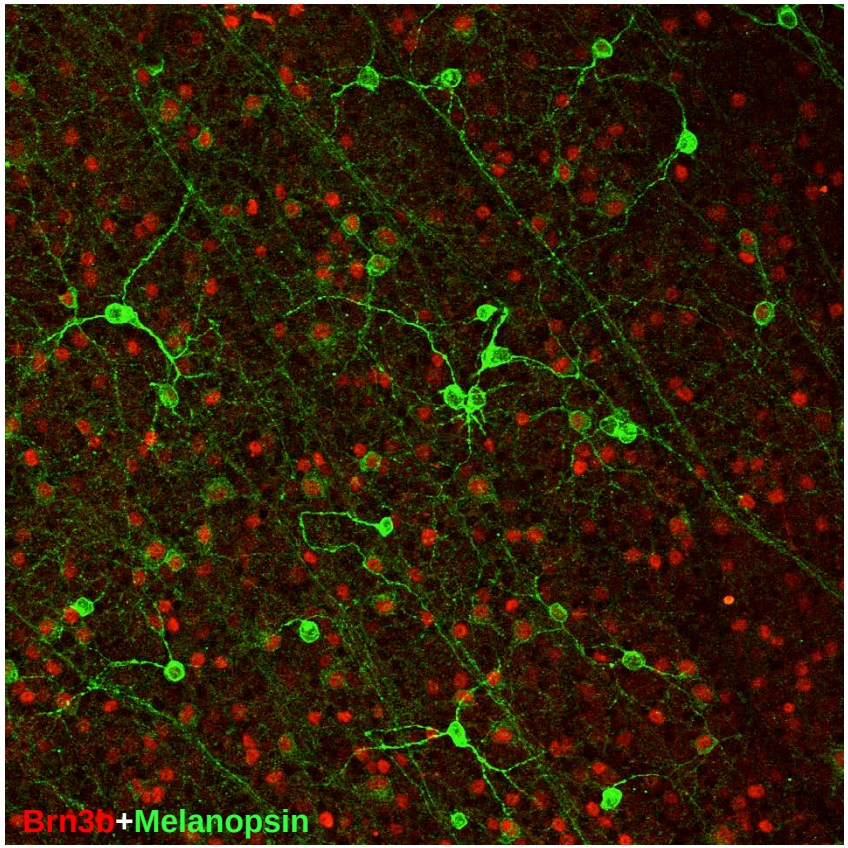


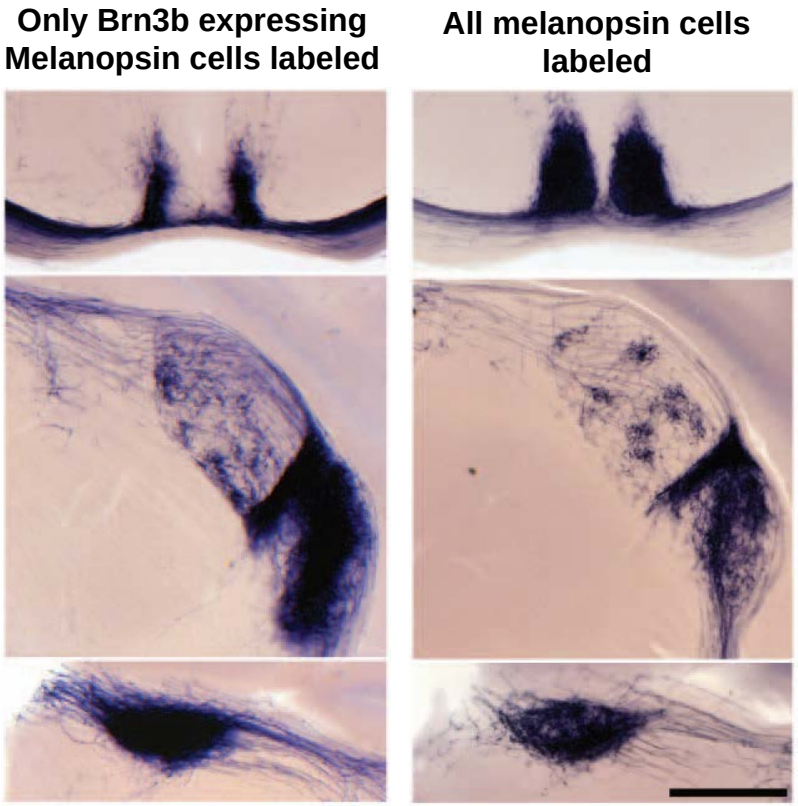
Ecker et al., Neuron (2010) 67, 49-60

The use of *Opn4*^{cre} mice, where the Cre recombinase gene is knocked into the melanopsin gene sequence, has revealed new types of ipRGC (M4 and M5), together with more extensive projections to other brain regions than was previously seen with the *Opn4*$^{tau\text{-}LacZ}$ mouse (see panel on right for comparison). The most interesting of these are the dorsal Lateral Geniculate Nucleus (dLGN) and superior colliculus (SC).

The panel to the left shows electrophysiological recordings from single M1, M2 and the new M4 type of ipRGCs. Note the large dendritic field and small intrinsic light response of M4 cells.

An increasing diversity of ipRGCs…

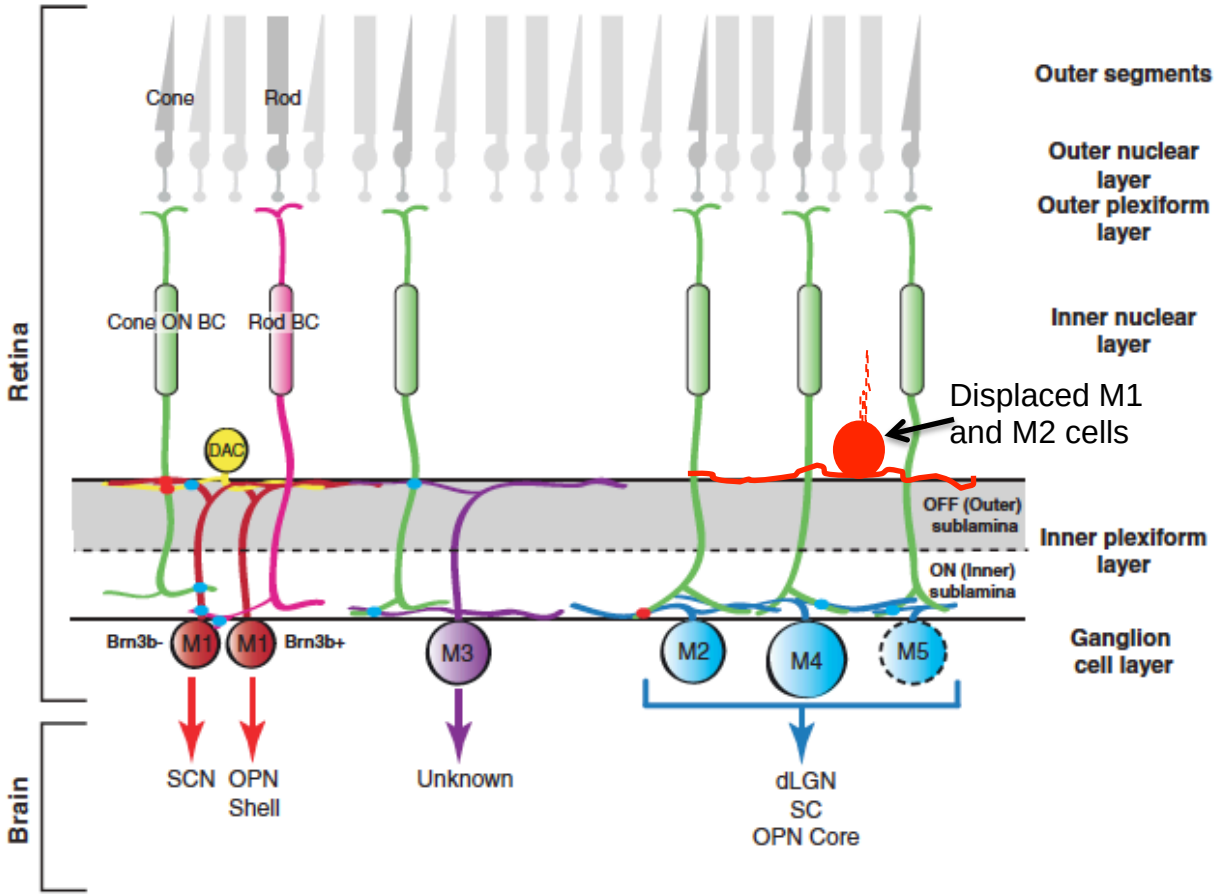


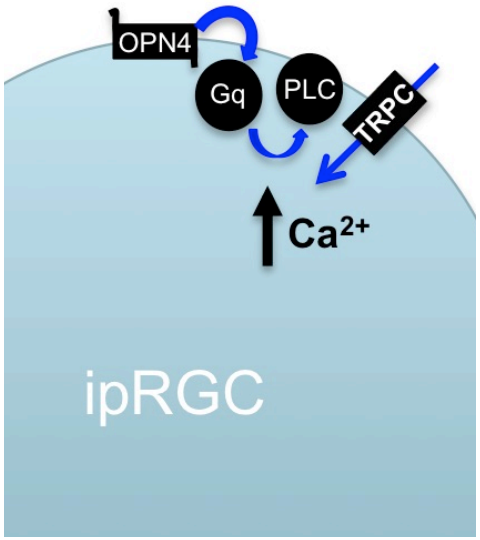


Some M1 type ipRGCs are negative for the transcription Factor Brn3b. It has been found using transgenic reporter mice that these Brn3b negative M1 ipRGCs (approximately 200 cells) innervate the SCN and are sufficient to drive circadian photoentrainment.

Chen et al., Nature (2011) 476, 92-95

Summary of different ipRGC subtypes and their sub-cortical projections.

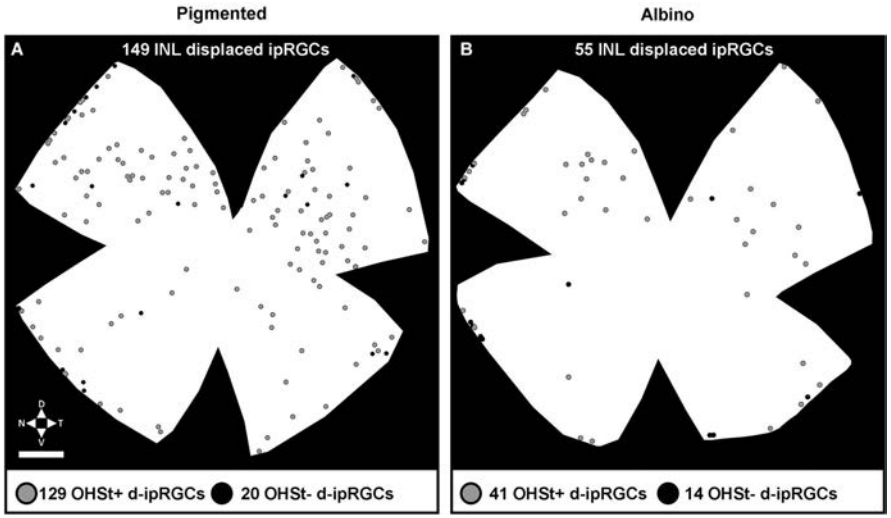


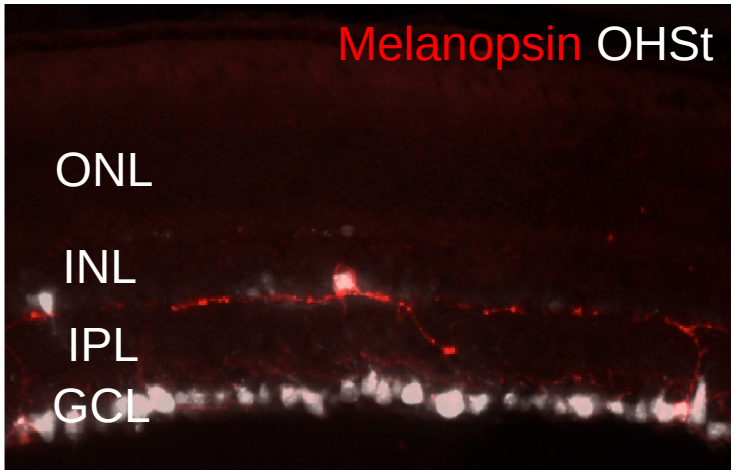


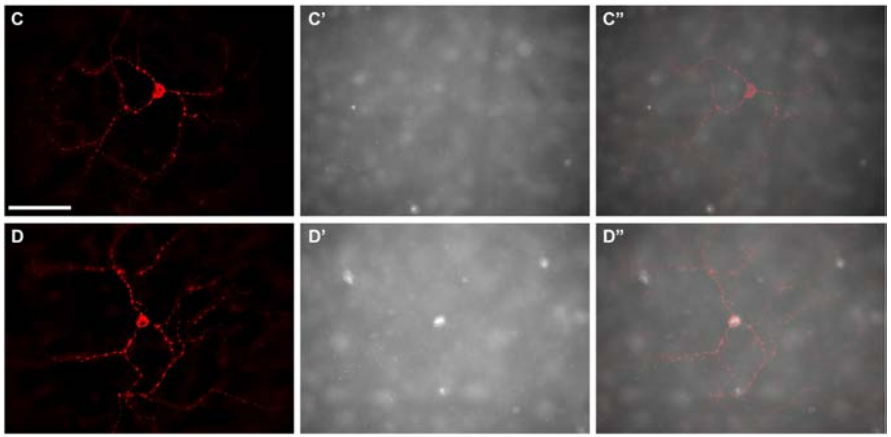
The phototransduction signaling cascade used by ipRGCs is “invertebrate-like”

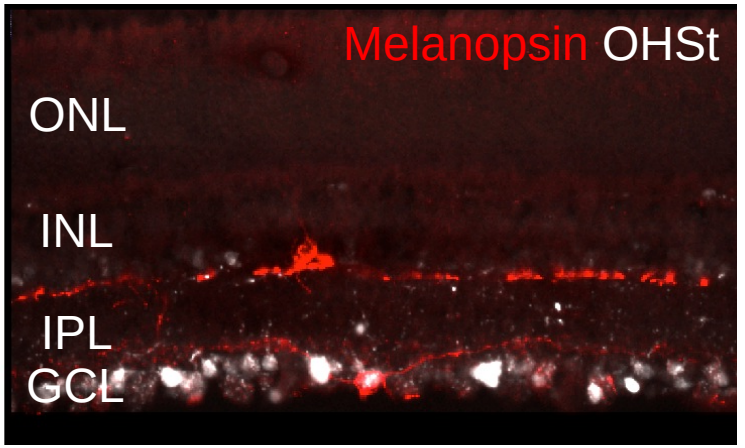
(Schmidt et al., TINS (2011) 34(11) 572-80)

Some ipRGCs are displaced to the INL and ~14% of these also lack an axon to the brain









Retrograde tracer (OHSt) was applied to the severed optic nerve head.

Valiente-Soriano et al., Front. Neuroanat. (2014) 8, 131.

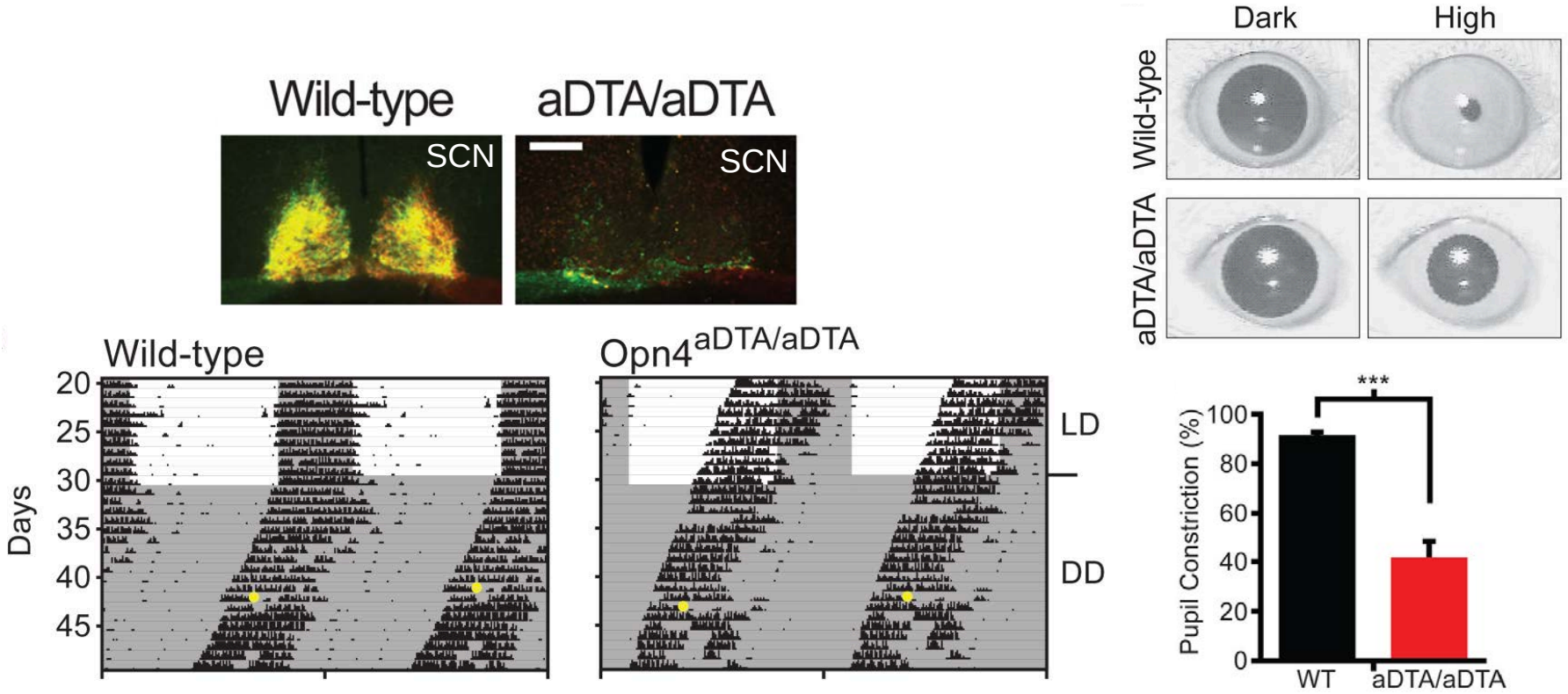
Overview:

- The discovery of intrinsically photosensitive Retinal Ganglion Cells (ipRGCs)
- Structure and function of ipRGCs
  - Anatomy and physiology
  - How do ipRGCs contribute to visual function?

Visual function

- Vision is often referred to as:
  - “Non-image forming” vision
  - “Image-forming” vision
- Non-image forming vision
  - Circadian physiology (photoentrainment, pineal melatonin, body temperature).
  - Pupillary light reflex (PLR)
  - Light perception
- Image-forming vision
  - Brightness, acuity, contrast, motion
  - Spatial and temporal dimensions

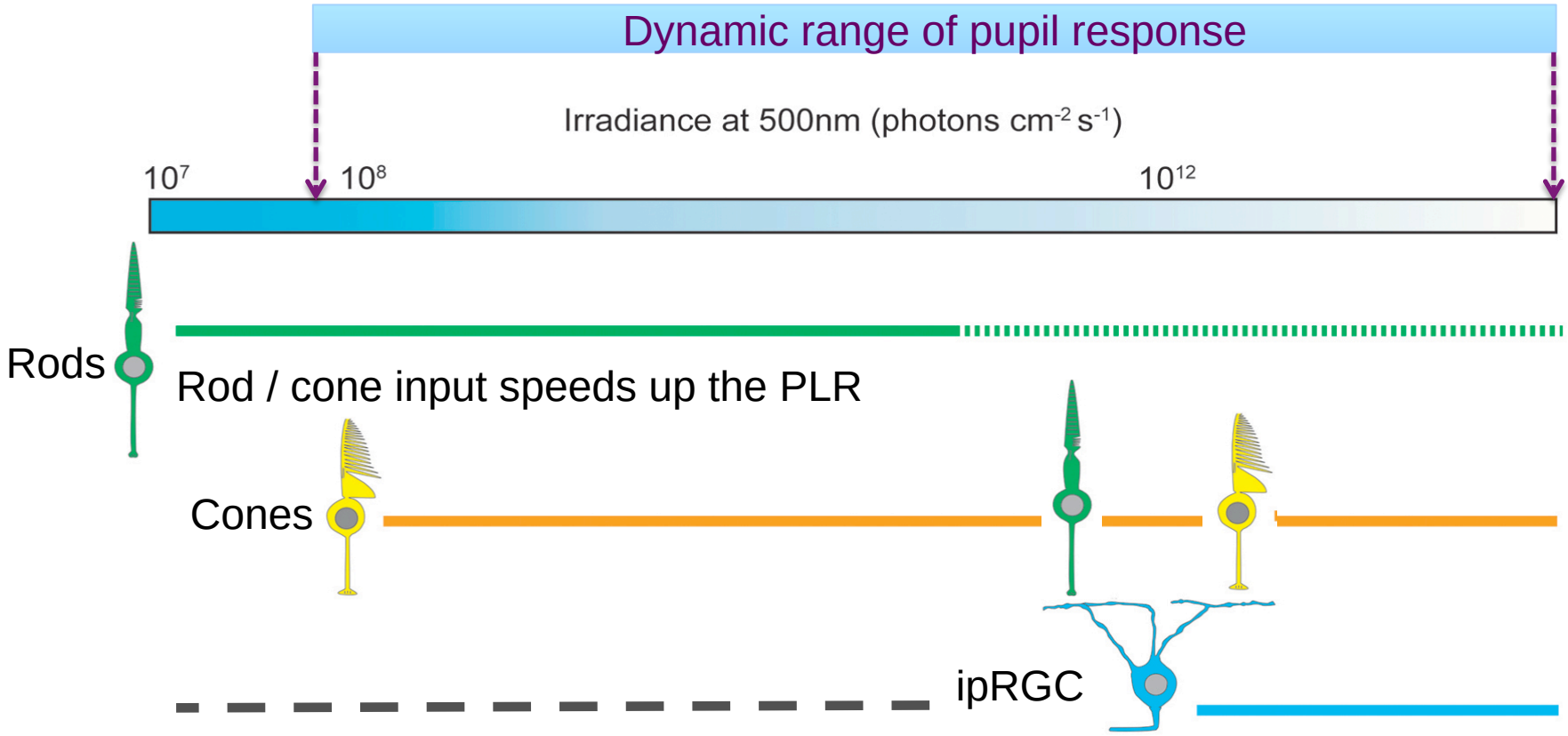
Genetic ablation of cells expressing melanopsin causes severe deficits in phase shifting and PLR



To physically remove melanopsin cells, the attenuated diptheria toxin A subunit was introduced into the mouse melanopsin gene locus. In mice homozygous for this mutation (aDTA/aDTA), there was a severe reduction in target innervation in subcortical brain regions, an abolition of circadian photoentrainment and only a 40% pupil constriction at the highest irradiances.

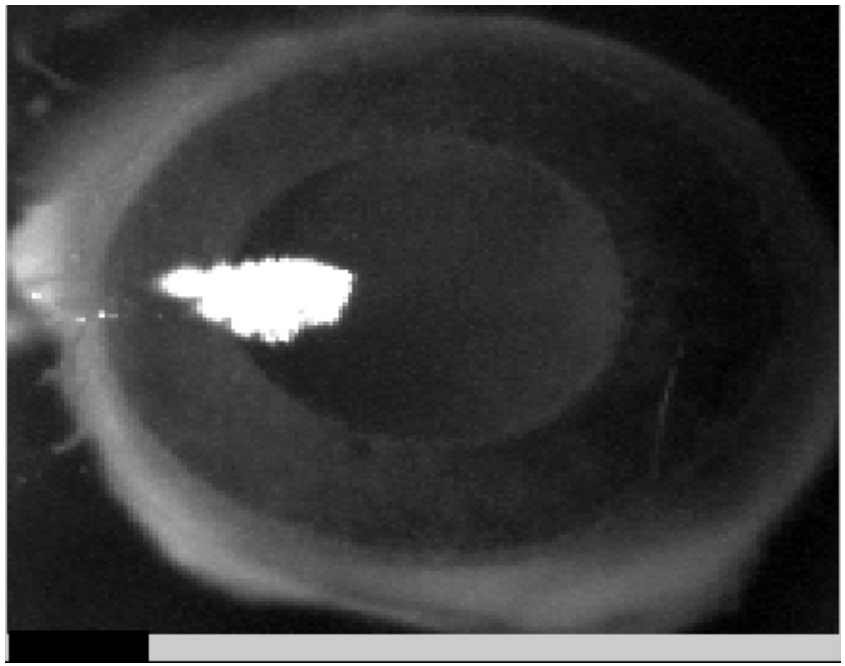
Guler et al., Nature (2008) 453, 102-5

Melanopsin sustains the PLR at high irradiance but ipRGCs signal over a wide dynamic range due to rod/cone input

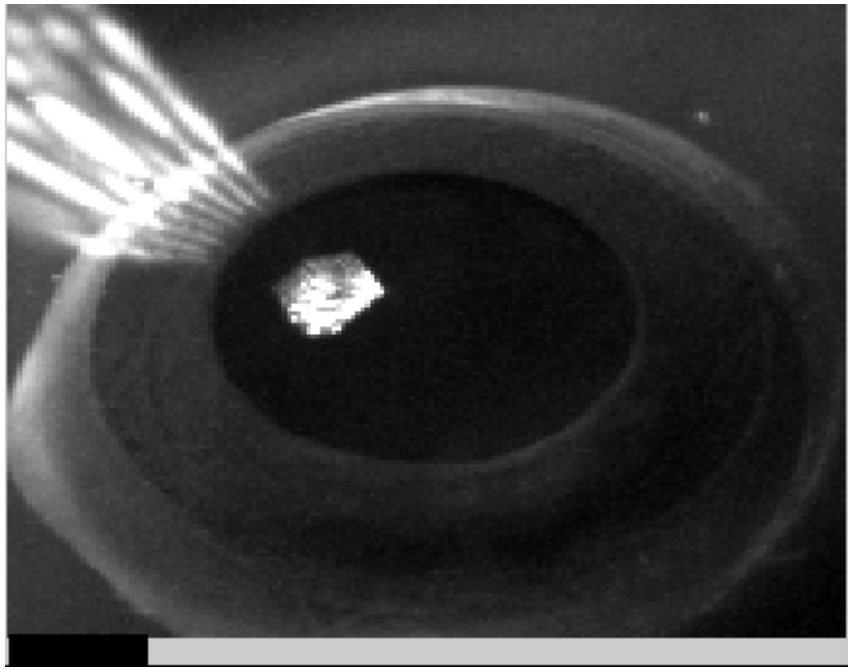


In rodents, melanopsin maintains pupil constriction independently of the brain!

Adult Wildtype mouse



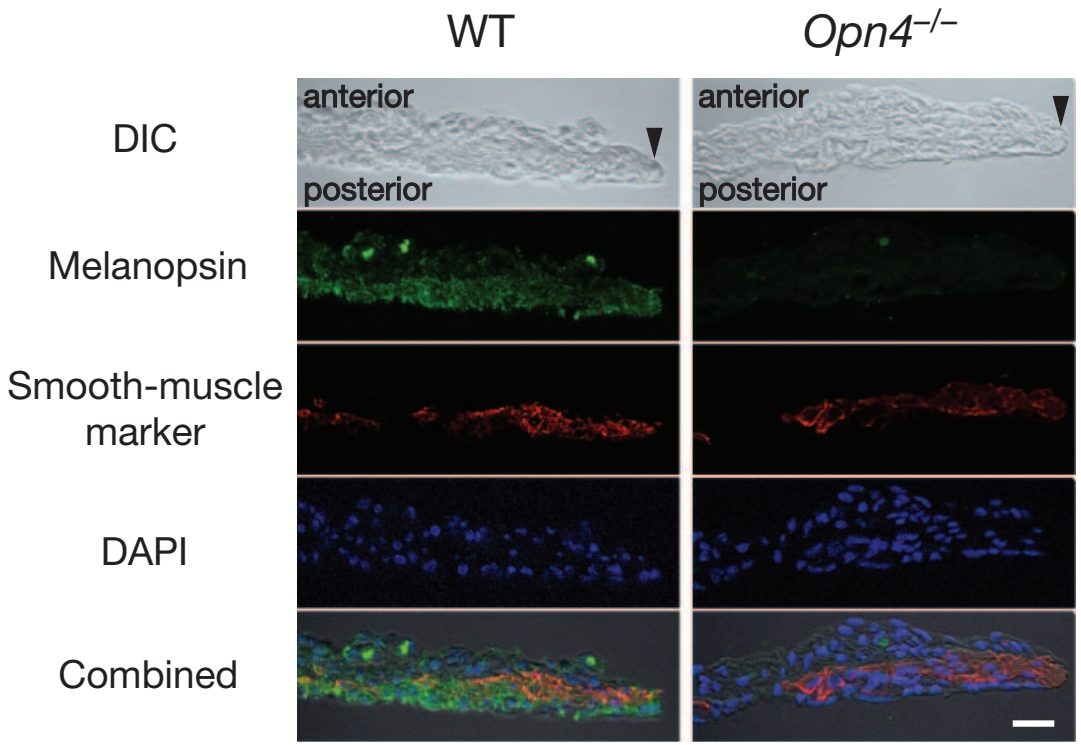
***Opn4*$^{-/-}$ mouse**



The intrinsic PLR (iPLR) is slower than the conventional PLR.

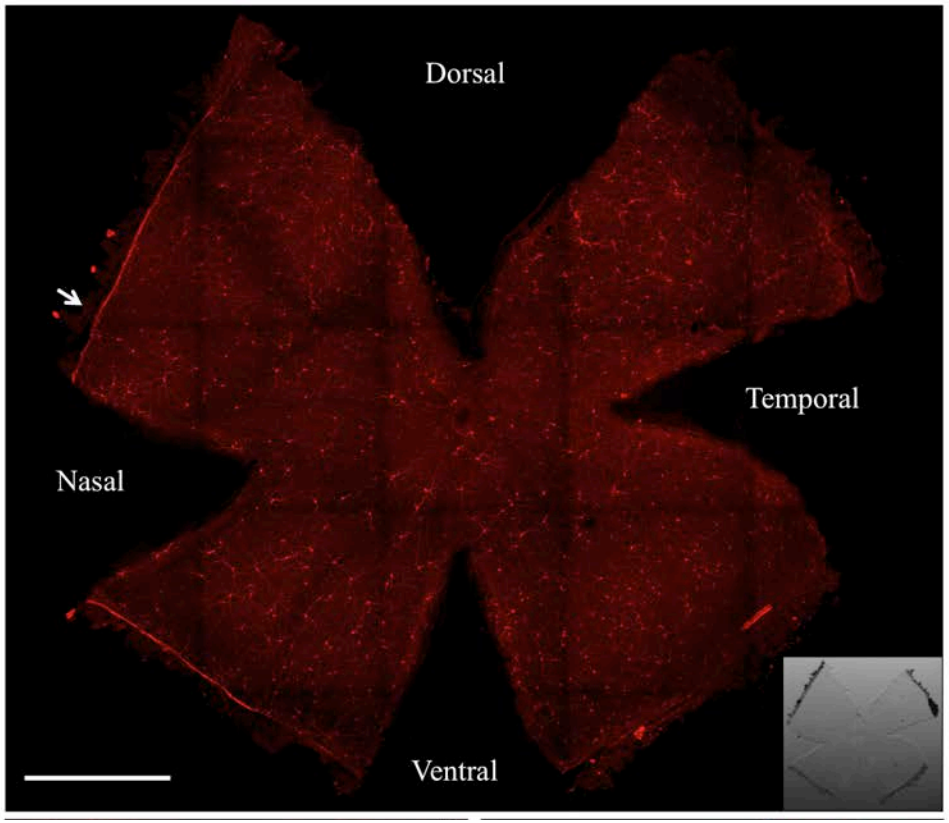
Xu et al., Nature (2011) 479: 67-73
Semo et al., Experimental eye research (2014) 119: 8-18
Vugler et al., Neuroscience (2015) 286 60-78

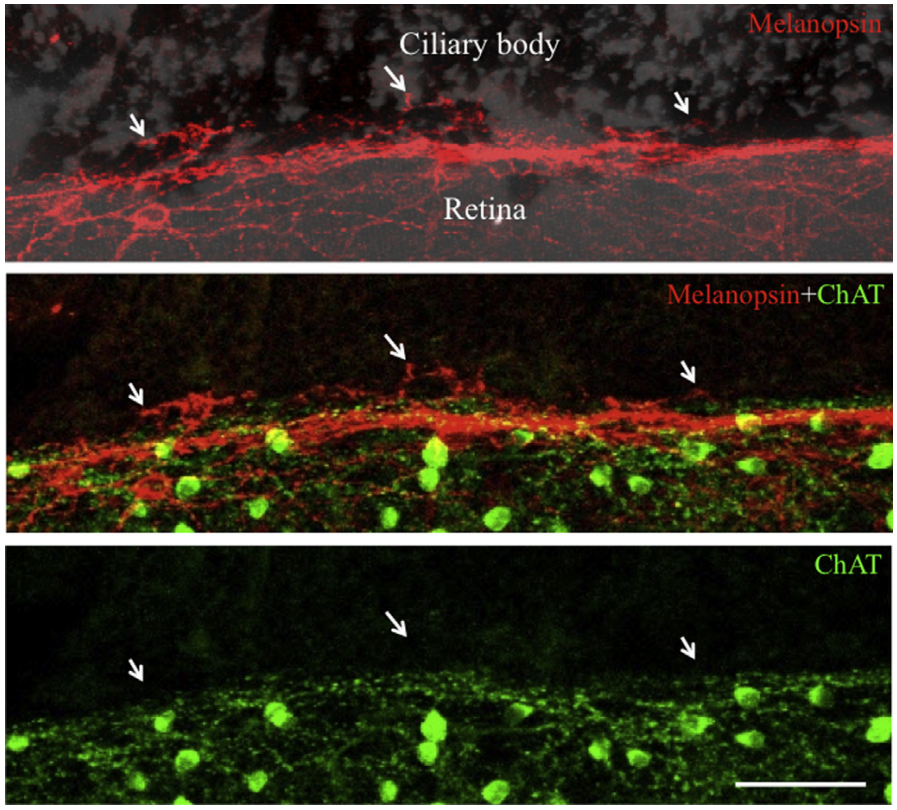
The intrinsic PLR (iPLR) is thought to involve melanopsin in the iris



Iris sphincter muscle of the albino mouse eye
Stained for melanopsin and smooth muscle actin
(Xu et al., Nature (2011) 479: 67-73)

In mice, ipRGCs at edge of retina also send small projections into the ciliary body…

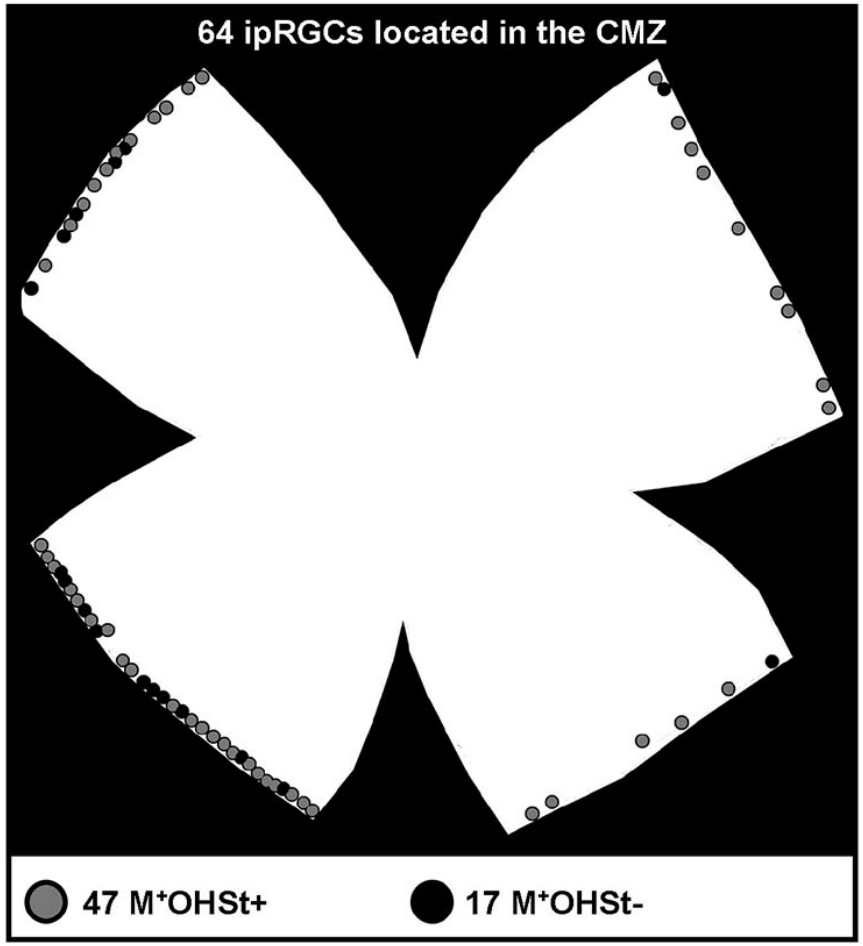


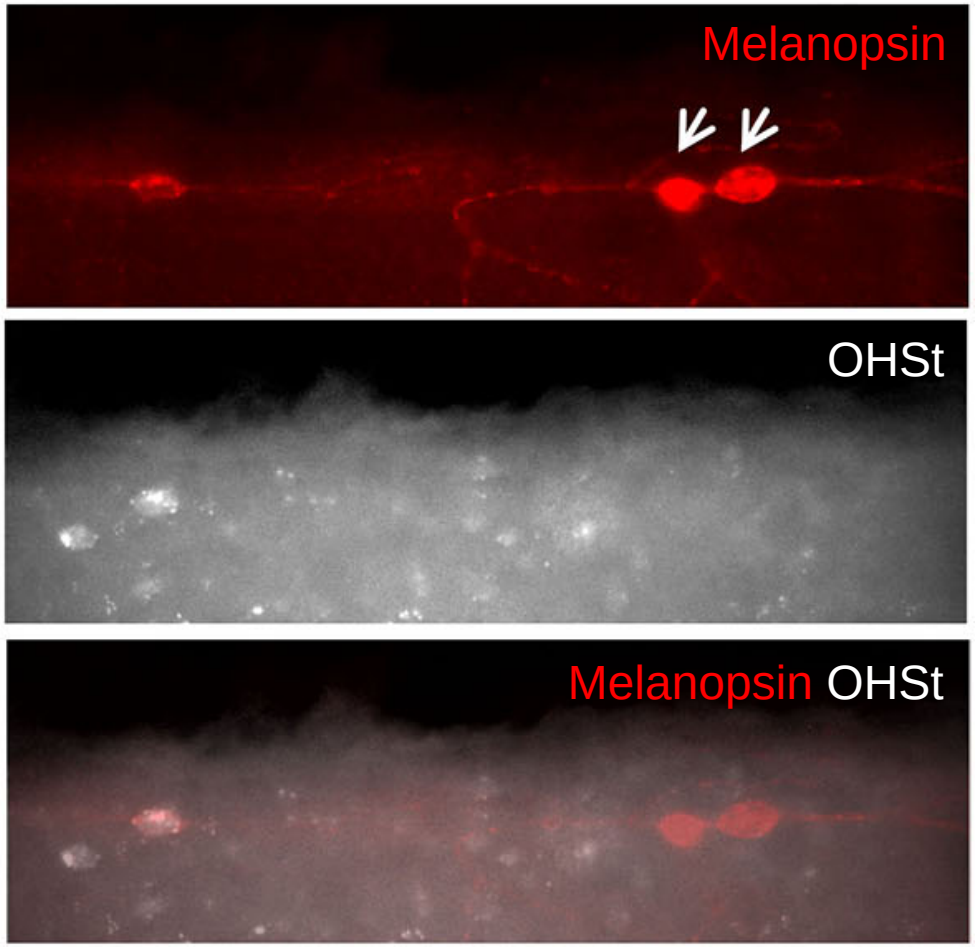


Melanopsin is also expressed at low levels in the ciliary body

Semo et al., Experimental eye research (2014) 119: 8-18

At the ciliary marginal zone (CMZ) ~20% of ipRGCs lack an axon to the brain

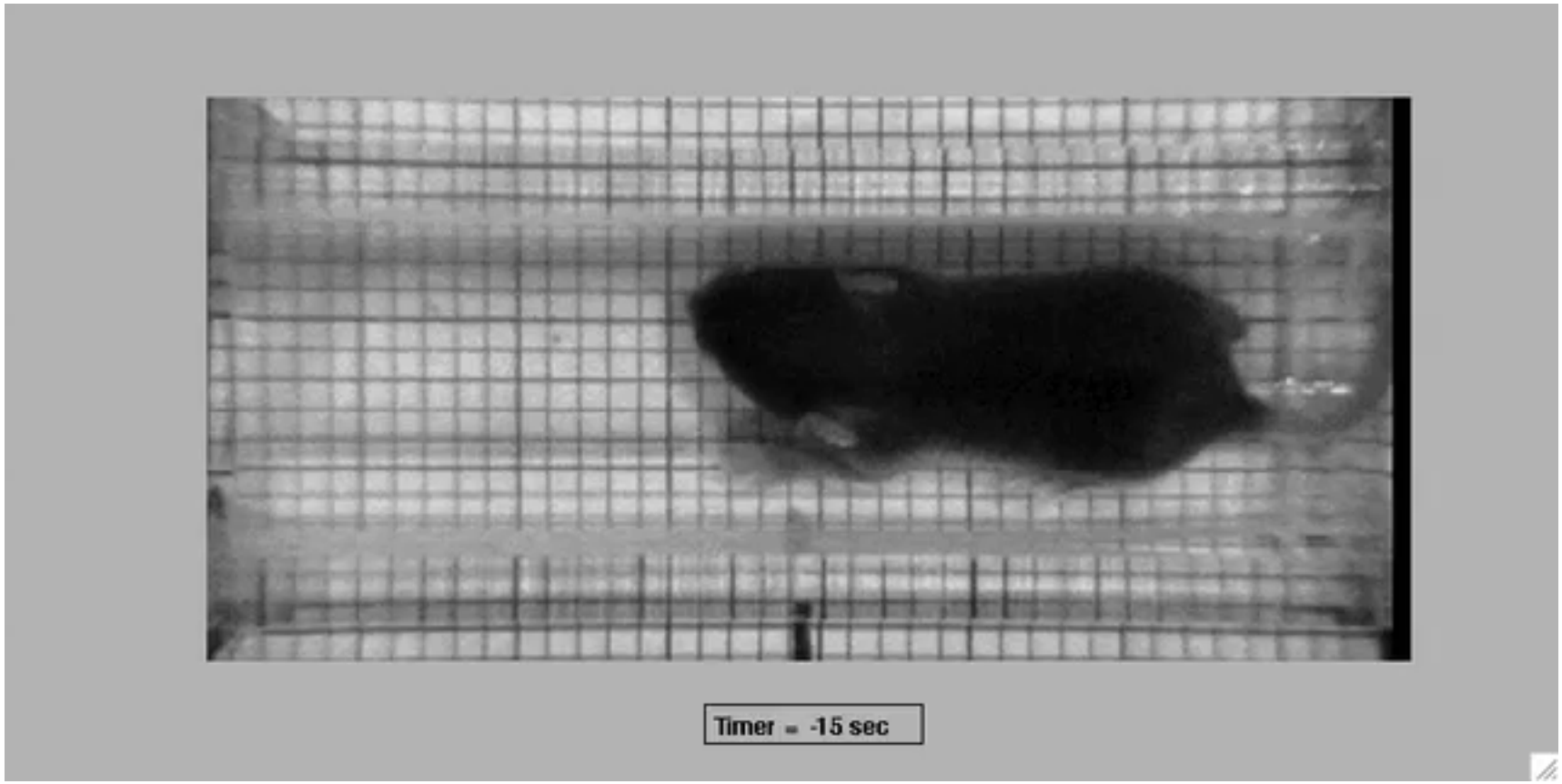




Demonstrated by application of the retrograde tracer OHSt to the severed optic nerve in wildtype pigmented mice.

Valiente-Soriano et al., Front. Neuroanat. (2014) 8, 131.

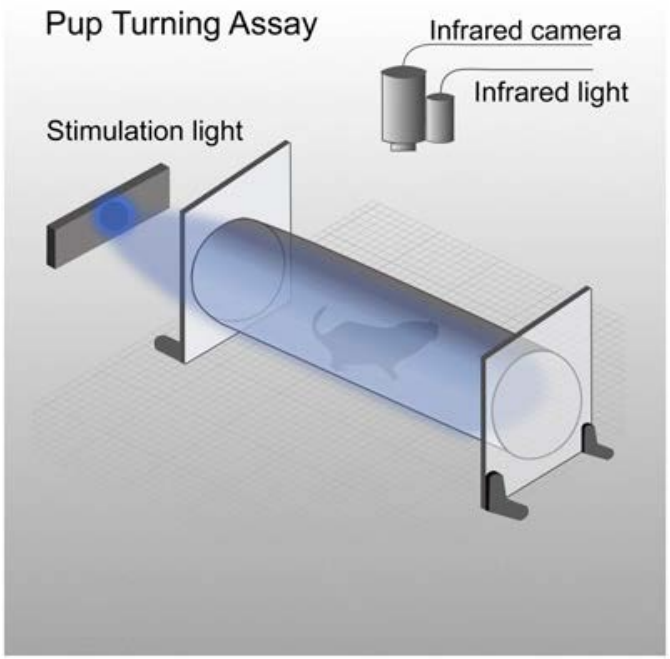
A role for melanopsin in light perception ?

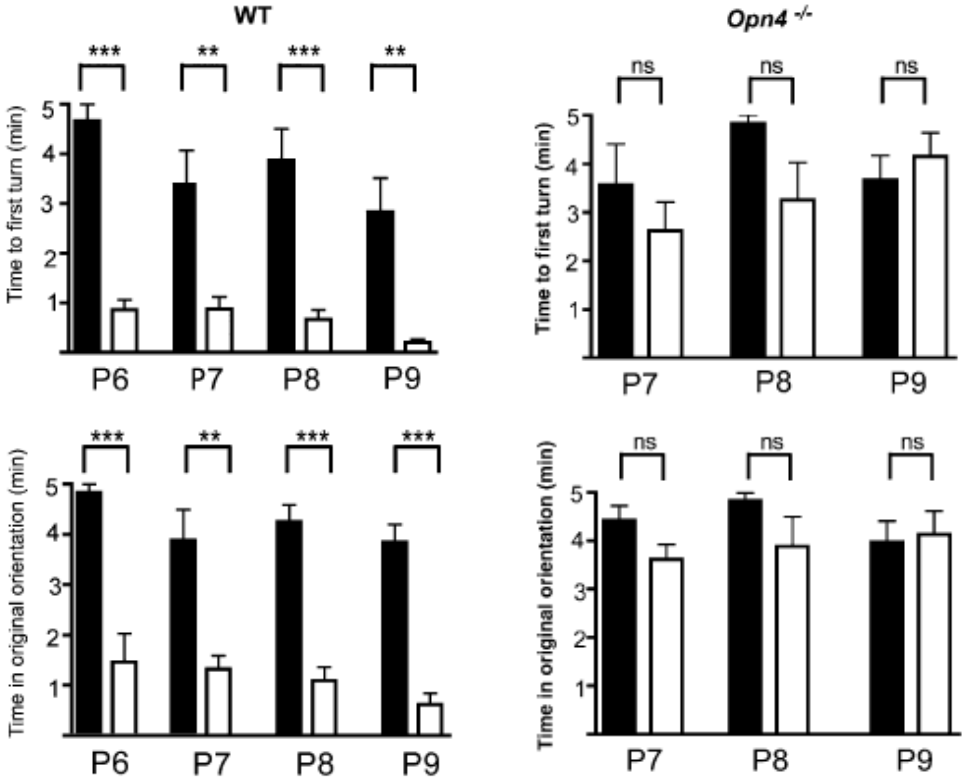


They used a turning assay to study light avoidence behaviour in neonatal mice (P6-P9)

(Johnson et al., *PNAS* (2010) 107(40) 17374-8)

Melanopsin is required for light avoidance behaviour in neonatal mice

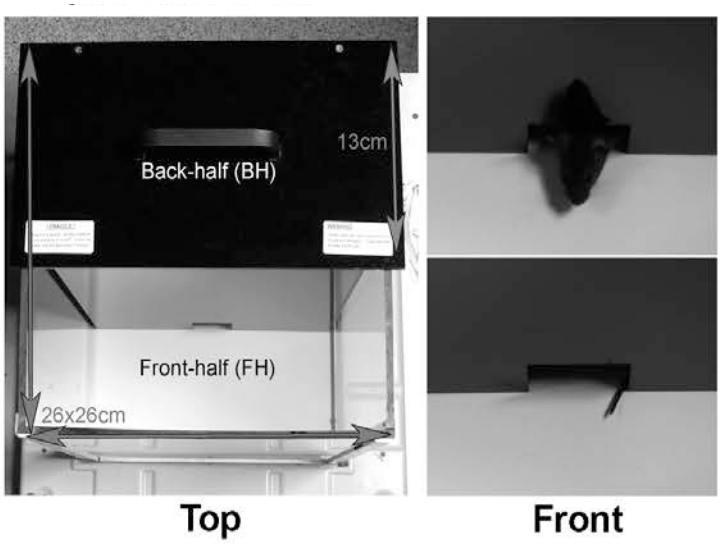




Neonatal mice were exposed to blue light (open bars) or darkness (black bars). In wildtype mice, the latency to their first turn in the experimental tube was significantly reduced by light exposure, as was the time spent in their original orientation. This was not the case for OPN4$^{-/-}$ mice. *** P<0.001, ** P<0.01, ns = non significant.

(Johnson et al., (2010) *Proc. Natl. Acad. Sci. USA* 107(40) 17374-8)

ipRGCs drive behavioural light aversion in adult mice

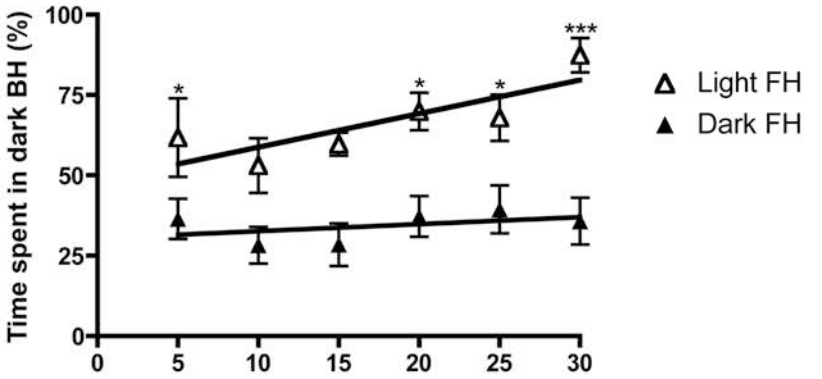


30-minute trial, 1300 lux light

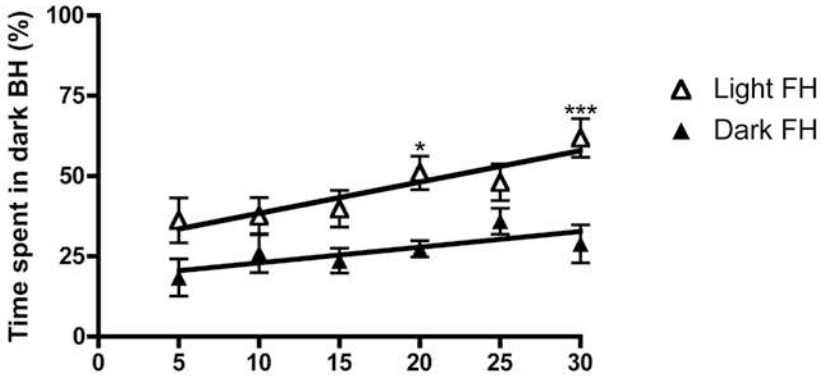
We used *rd/rd cl* mice (lacking rods and cones) to show that ipRGCs can mediate light aversion behaviour In adult mice. Interestingly, light aversion behaviour gets stronger as the trial progresses in *rd/rd cl* mice. *Opn4*$^{-/-}$ mice retain an aversion to light without the potentiation over time (not shown)

Semo et al., PLoS One. (2010) 5(11), e15009

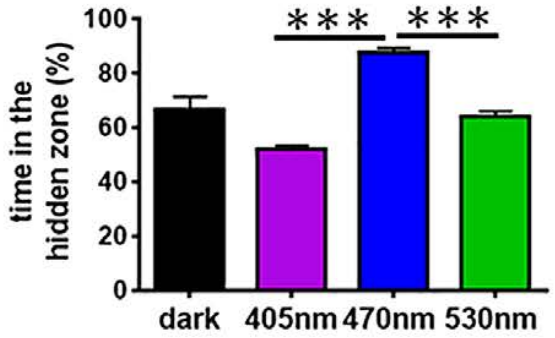
Wildtype mice

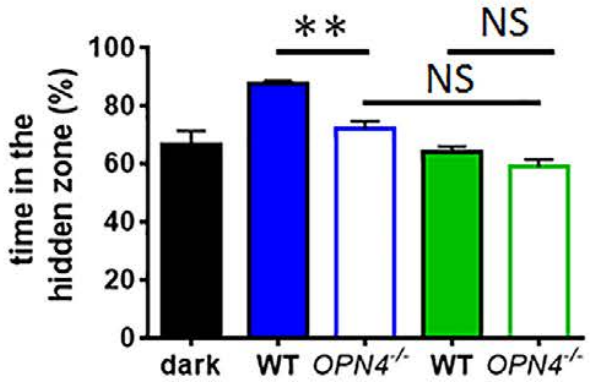


rd/rd cl mice



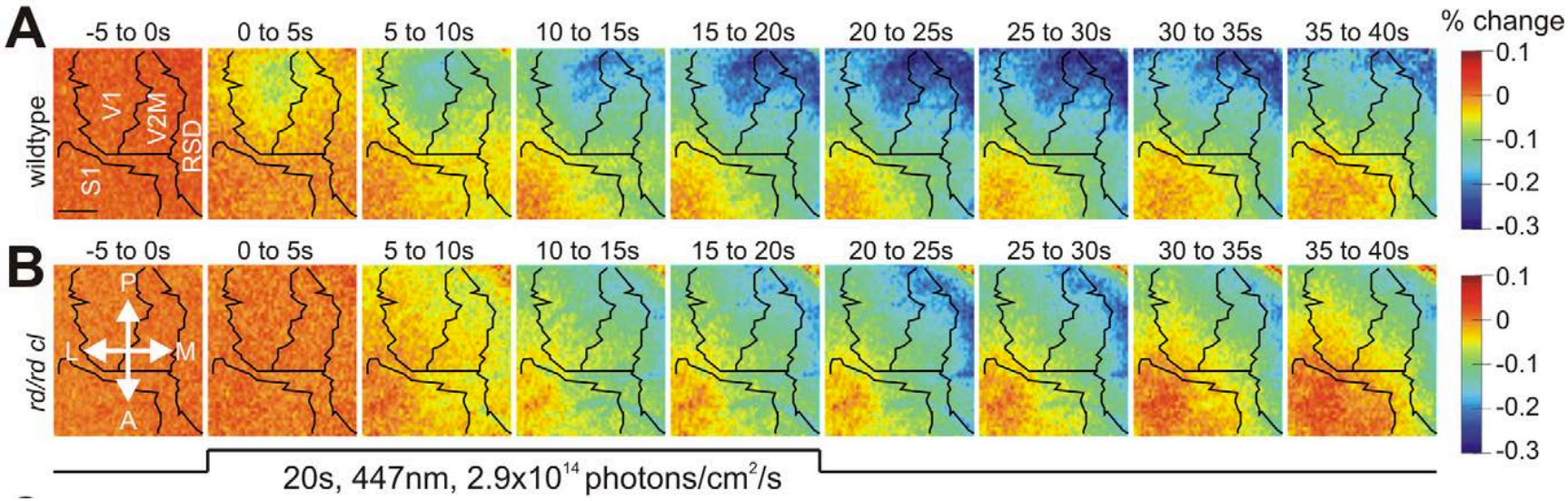
Blue light drives behavioural light aversion in adult mice





Pilorz et al., PLOS Biology, 2016 14(6): e1002482

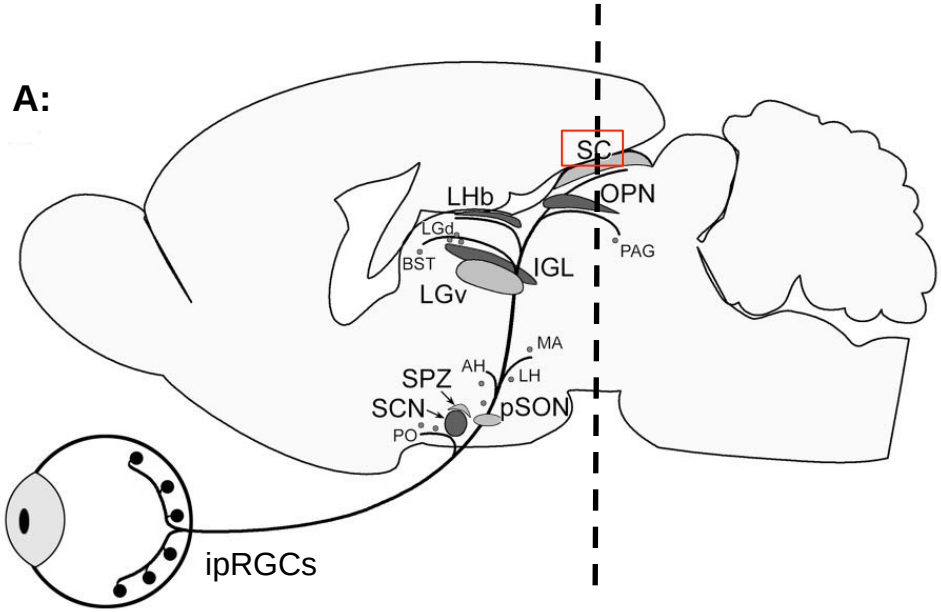
Intrinsic optical imaging reveals the dynamics of ipRGC-driven cortical activation in *rd/rd cl* mice

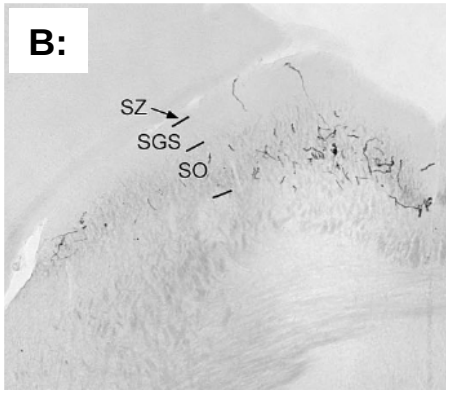


Intrinsic optical imaging signals from the visual cortex of wildtype and *rd/rd cl* mice exposed to a 20 second pulse of bright blue light. The time course before (-5 to 0s) and after light exposure is shown across the top. Blue indicates regions of most intense neural activity. Note that the activity begins in V1 and spreads across to retrosplenial visual cortex (RSD) in wildtype (WT) mice, while activation appears more slowly in *rd/rd cl* mice. Cortical activation in *rd/rd cl* mice has also been demonstrated using functional anatomy (c-fos staining).

Brown et al., PLoS Biology. (2010) 8(12), e1000558
Semo et al., PLoS One. (2010) 5(11), e15009

Light aversion may involve ipRGC input to both the Superior Colliculus and visual cortex





Superior Colliculus (SC)

The Superior Colliculus (SC) is a specialised region of the dorsal midbrain which co-ordinates basic movements / behaviour. as shown in the sagittal section (A), ipRGCs project to the SC. The image in B is a coronal section through the SC of a melanopsin-reporter mouse (Melanopsin$^{tau\text{-}LacZ/+}$).

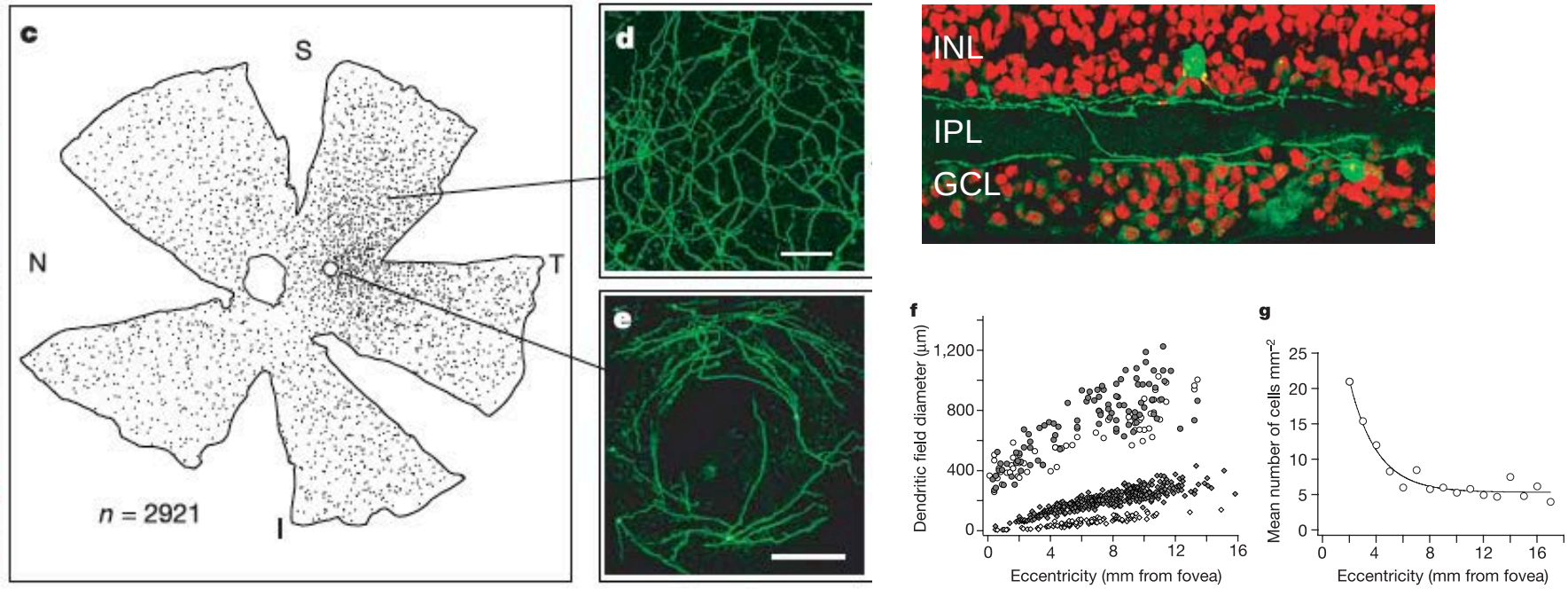
It has been reported that lesions to the SC in P5 neonatal rats can prevent the light avoidance response (Routtenberg et al., Developmental Psychobiology (1978) 11(5): 469-478).

However, light aversion behaviour is also impaired in adult rats with lesions to visual cortex (Altman, Am J. Physiol (1962) 202: 1208-1210).

A role for melanopsin in the conscious perception of light in humans?

- Three main studies to date:
  - ipRGCs project to the dLGN in primates (Dacey et al., Nature (2005) 433(17) 749-54).
  - Humans lacking rods / cones can perceive blue light (Zaidi et al., Current Biology (2007) 17 2122-28) and experience photophobia (Noseda et al., Nature Neuro. (2010) 13(2) 239-45).
  - Psychophysical tests in human subjects suggest a role for melanopsin in brightness discrimination (Brown et al., Current Biology (2012) 22, 1-8).
- Does melanopsin contribute to image-forming vision?
  - Acuity? Contrast? Brightness? Motion?

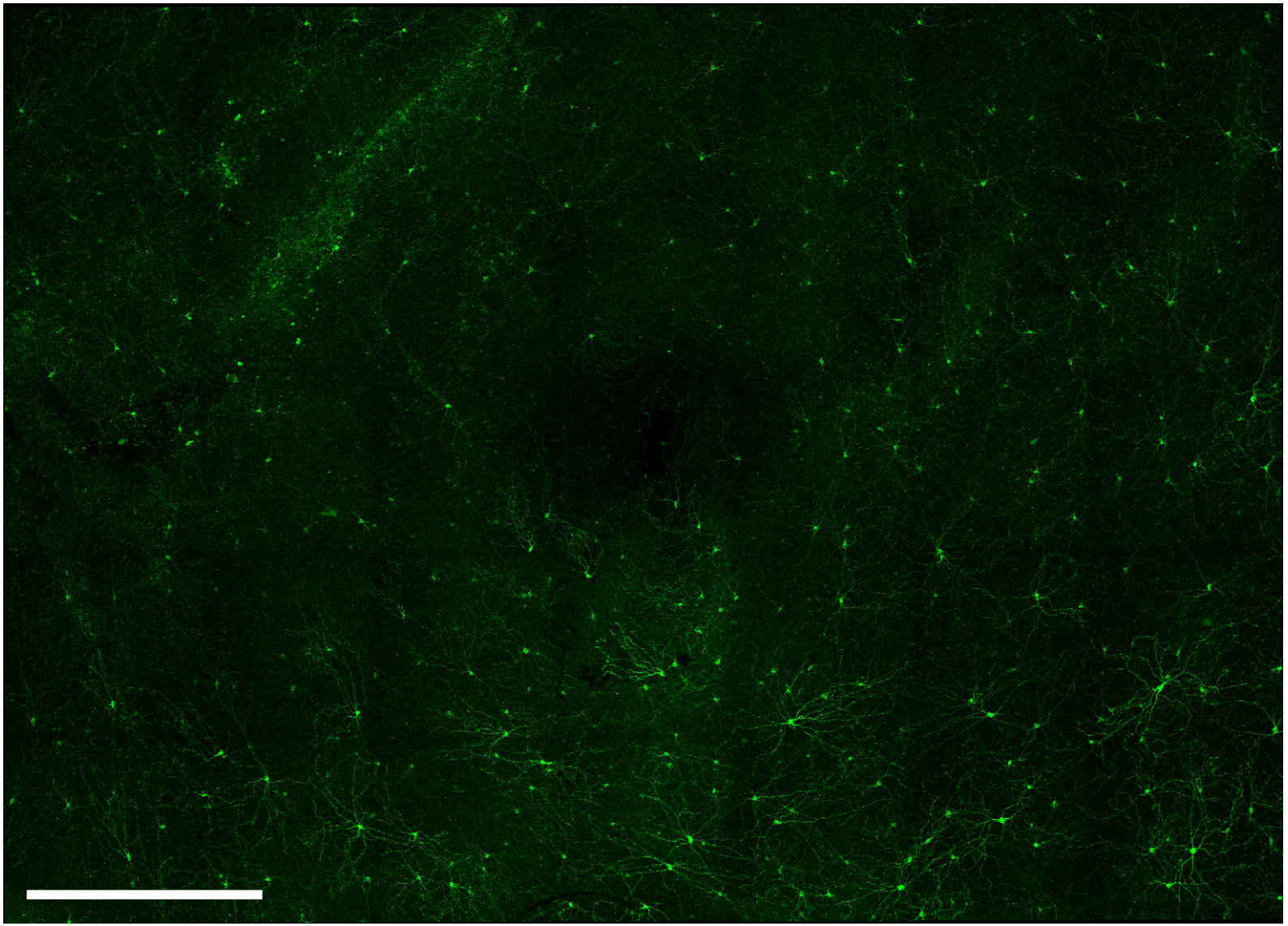
In primates, ipRGCs increase in density towards the fovea and project to dLGN



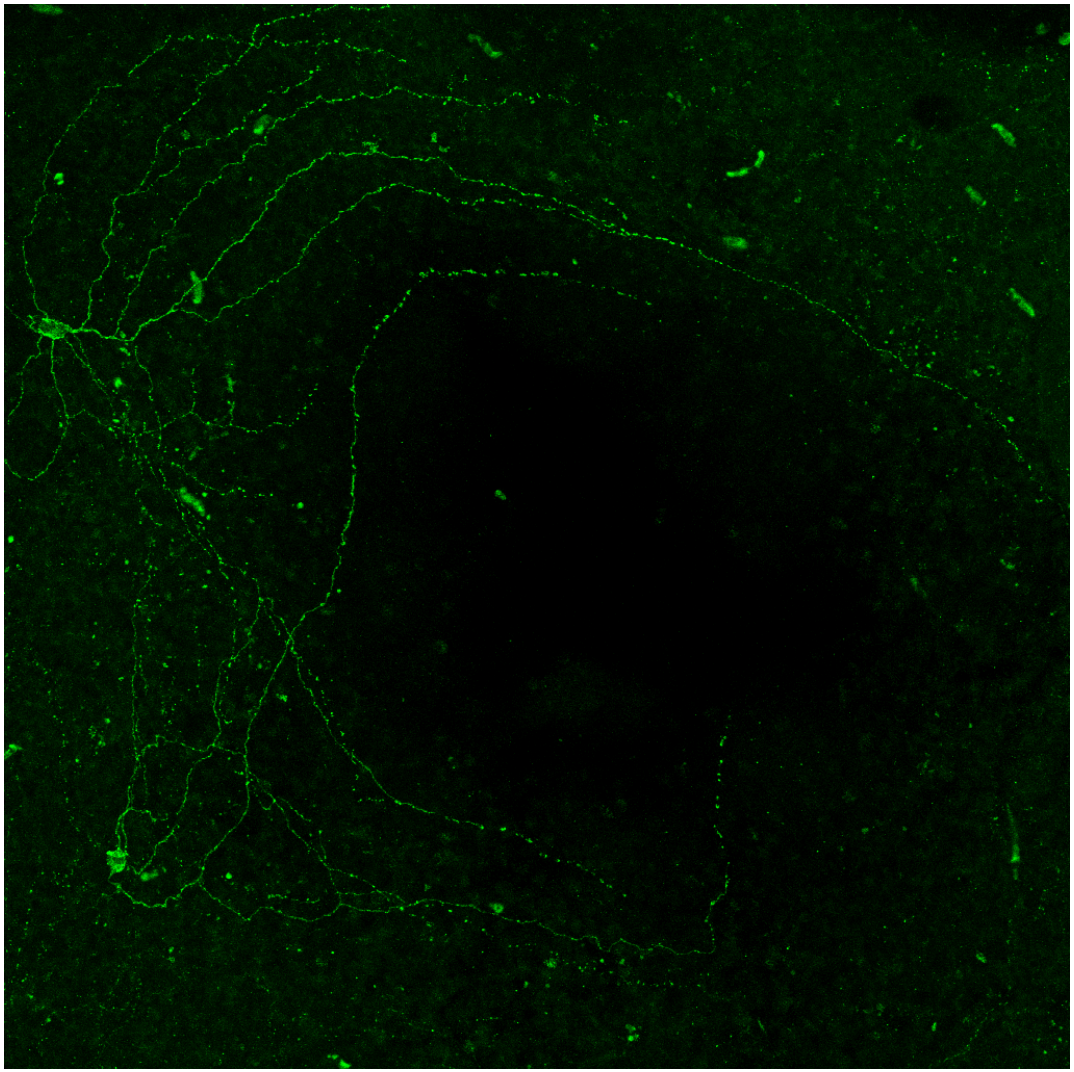
A study by Dacey and colleauges has shown that intrinsically photosensitive melanopsin positive ganglion cells project to the dLGN and are strongly activated by rods and cones. The receptive field of these cells displays colour opponency (S-OFF, L+M-ON). In contrast to rodents, the primate retina has a high percentage (40%) of melanopsin ganglion cells displaced to the inner nuclear layer (INL).

(Dacey et al., Nature (2005) 433(17) 749-54)

Distribution of ipRGCs in the human macula



ipRGCs penetrate the human fovea

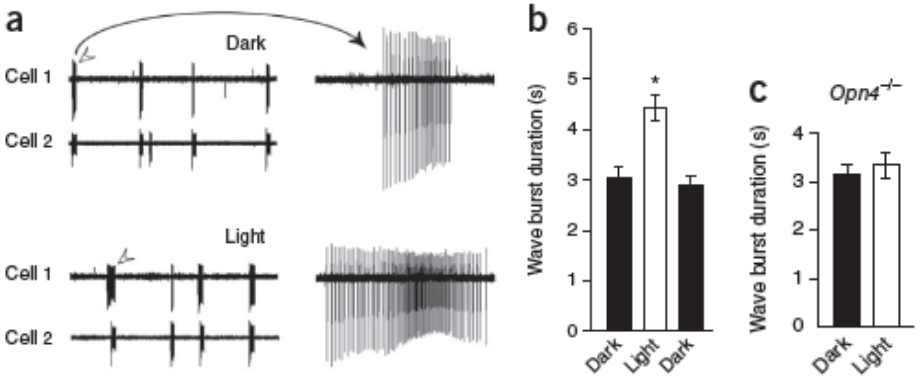


A role for melanopsin in contrast detection?

- Melanopsin is found in ON alpha RGCs and has been proposed to contribute to contrast detection
  - These RGCs are sensitive to contrast change signaled by rods/cones
  - *Opn4*$^{-/-}$ *mice* have behavioural deficits in contrast sensitivity
  - Schmidt et al. Neuron (2014) 82: 781-788.

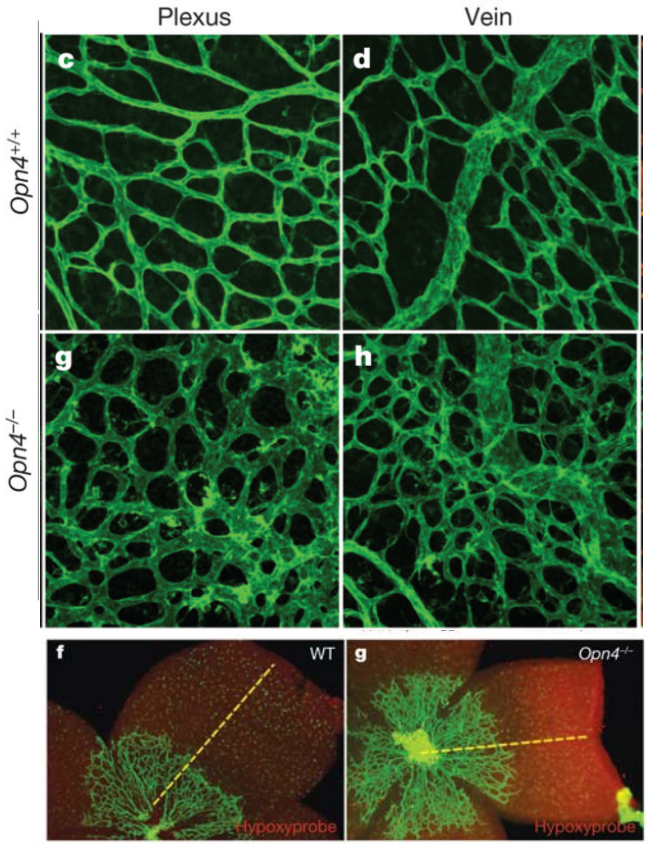
- However, studies looking at visual acuity and contrast sensitivity in *Opn4*$^{-/-}$ mice assume that the visual system develops normally in these animals…

Visual system development is abnormal in melanopsin knockout (*Opn4*$^{-/-}$) mice



Light increases the duration of retinal waves (bursts of spiking activity) in conventional retinal ganglion cells (**a**). This was not the case *Opn4*$^{-/-}$ mice (**b**). These mice also have a reduction in the segregation of ipsilateral and contralateral pathways in the retinogeniculate pathway.

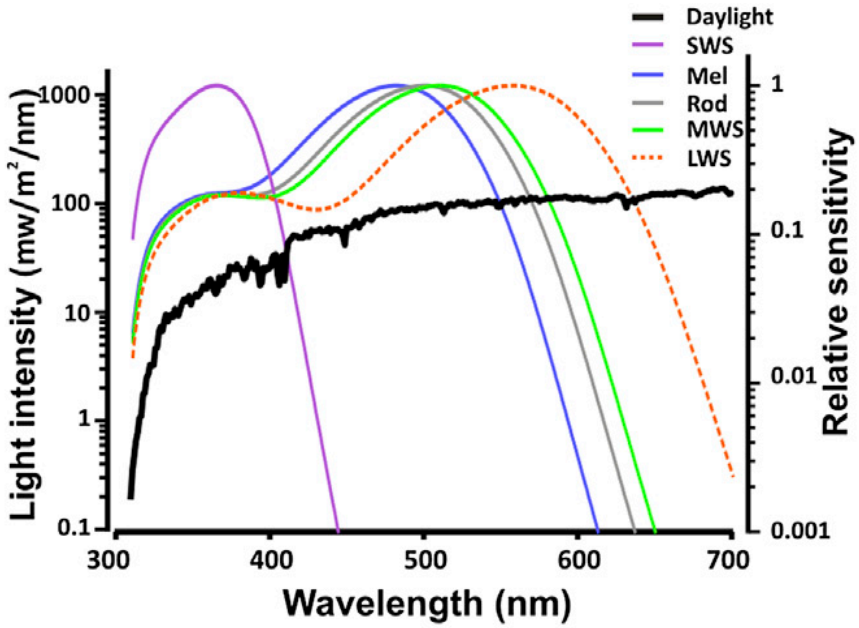
Rena et al., Nature Neuroscience. (2011) 14(7) 827-829).



Vascular abnormalities and hypoxia in the retina of young *Opn4*$^{-/-}$ mice. Too many neurons… (Rao et al., Nature (2013) 494(7436) 243-246)

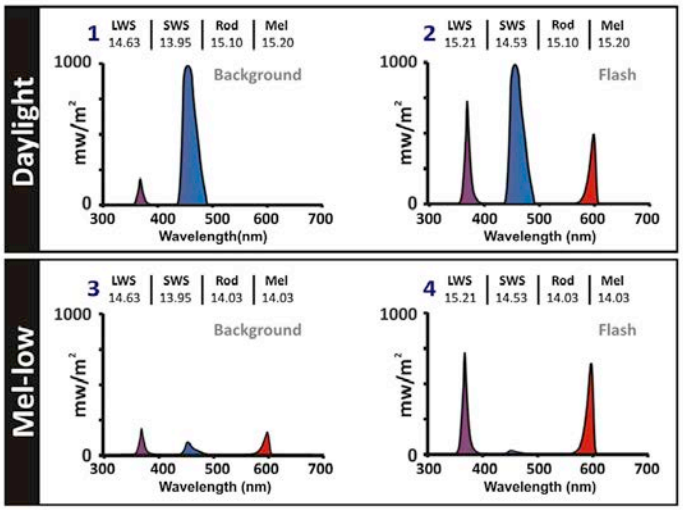
Selectively stimulating the melanopsin system in mice with an intact retina

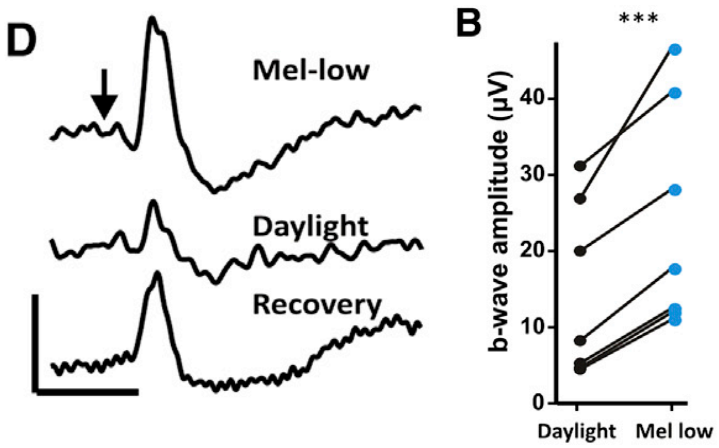
Very difficult to stimulate melanopsin without stimulating the green cones in normal mice. So, to make this possible, Rob Lucas' group in Manchester use red cone knock in mice (*Opn1mw*^{R}), where the mouse green cones express the human L-opsin (action spectrum shifted to the right and therefore enables a greater separation between melanopsin and green cone activation in mice).



This method was first used to demonstrate melanopsin-mediated brightness discrimination in intact mice (Brown et al., Current Biology (2012) 22, 1-8).

Melanopsin-driven light adaptation in mice





Melanopsin stimulation adapts the cone ERG in mice (200 x 1Hz, 50ms with Background illumination). Allen et al., Current Biology (2014) 24: 1-10

The *Opn1mw^{R} mouse was used to c*ompare retinal and thalamic responses to stimuli with spectral compositions either enriched (daylight) or depleted (Mel-low) in wavelengths to excite the melanopsin system.

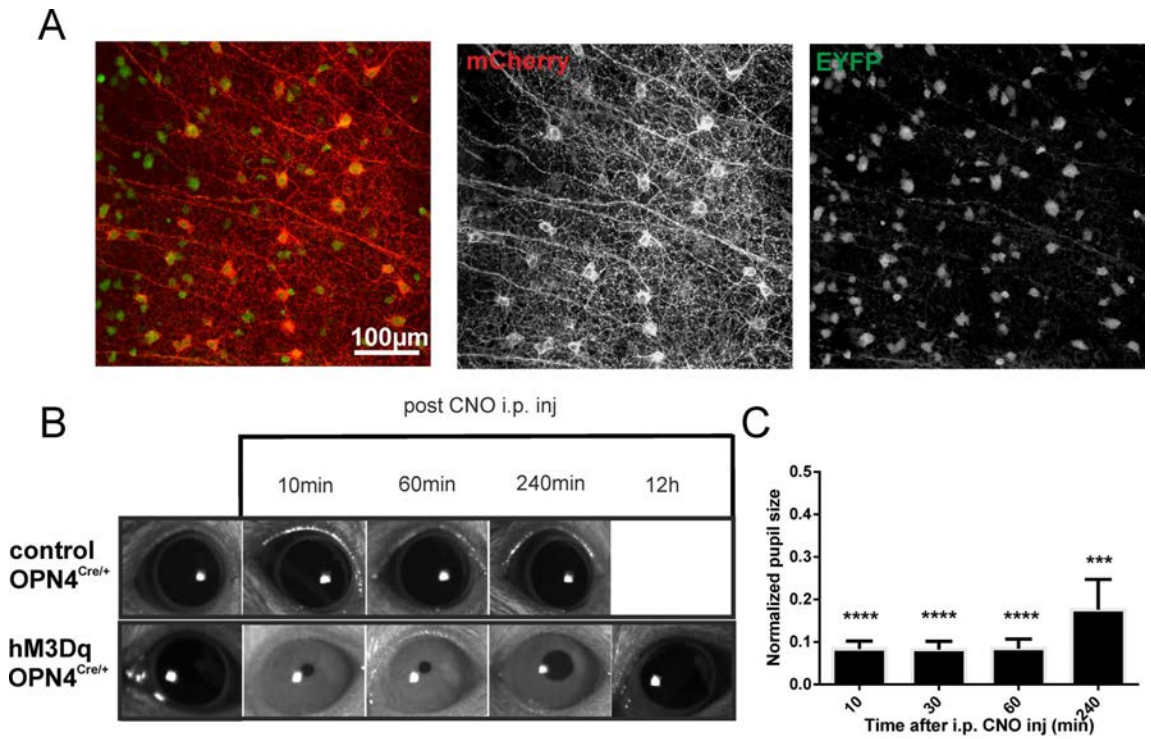
They found adaptation in the ERG (**D**&**B**) under daylight conditions and strong evidence for an increase in feature selectivity of dLGN neurons using multi-electrode recordings from this structure.

In the dLGN, the neurons preferred **finer spatial patterns** under daylight conditions. These conditions also tuned direction sensitive neurons to **faster motion**. So, increasing the level of melanopsin stimulation changes the feature detection of visual circuits in both spatial and temporal dimensions.

“**Melanopsin works like a photographer's light meter, providing an independent measure of irradiance to determine optimal settings for visual circuits.”**

At least part of this melanopsin-driven light adaptation may happen in the retina…

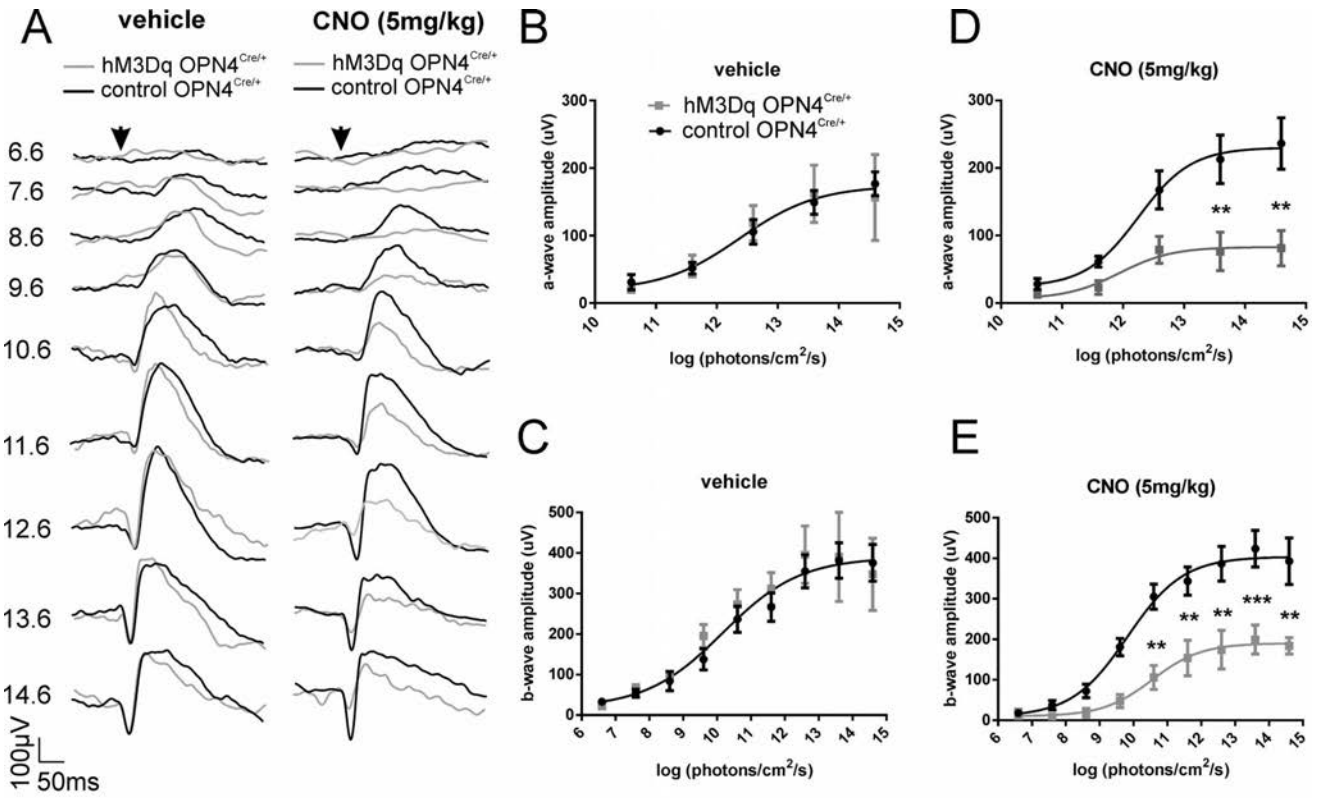
Chemogenetic activation of ipRGCs



The ipRGCs in OPN4-CRE mice can be selectively transfected by intravitreal eye injections of an AAV2 viral vector encoding the hM3Dq Gq-coupled receptor (which depolarises cells upon activation). The ipRGCs can then be selectively activated by intraperitoneal injections of Clozapine *N*-Oxide (CNO) in the dark. The images in **A** show Melanopsin expressing ipRGCs (green) transfected with hM3Dq (visualised in red using the mCherry reporter). In the dark, application of CNO causes sustained pupil constriction in conscious mice (B-C).

Milosavljevic et al., 2016, IOVS, 57(14):6305-12

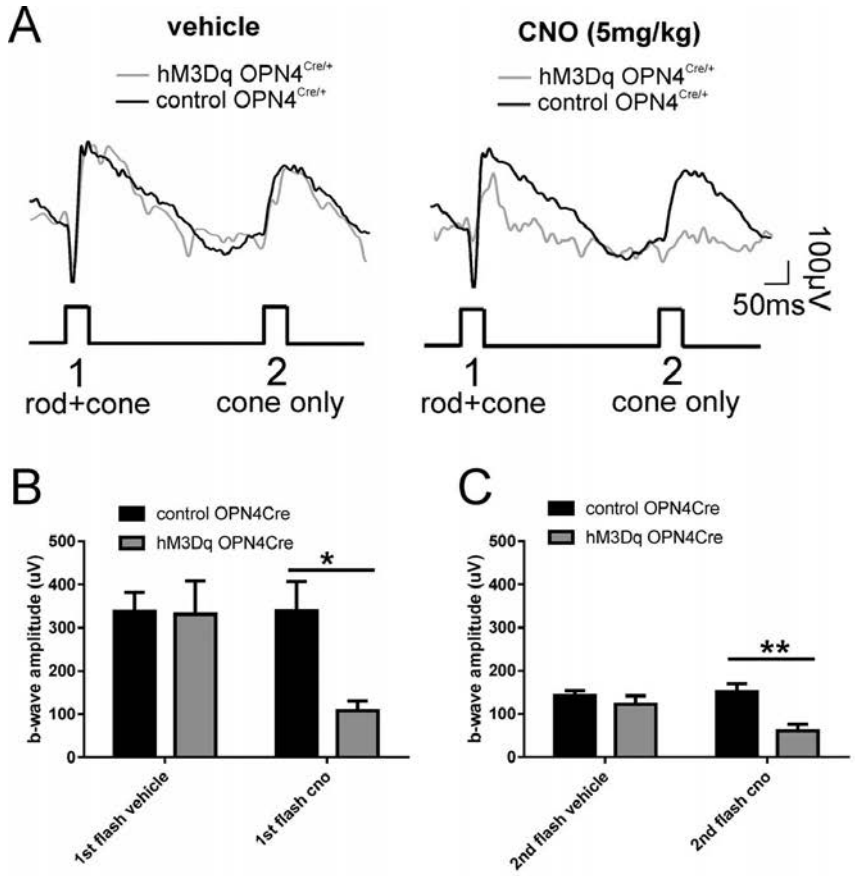
Chemogenetic activation of ipRGCs - Retina



ipRGC activation by CNO injection in hM3Dq OPN4-Cre mice reduces the amplitude of a and b-waves in the scotopic (dark-adapted) electroretinogram (ERG).

Milosavljevic et al., 2016, IOVS, 57(14):6305-12

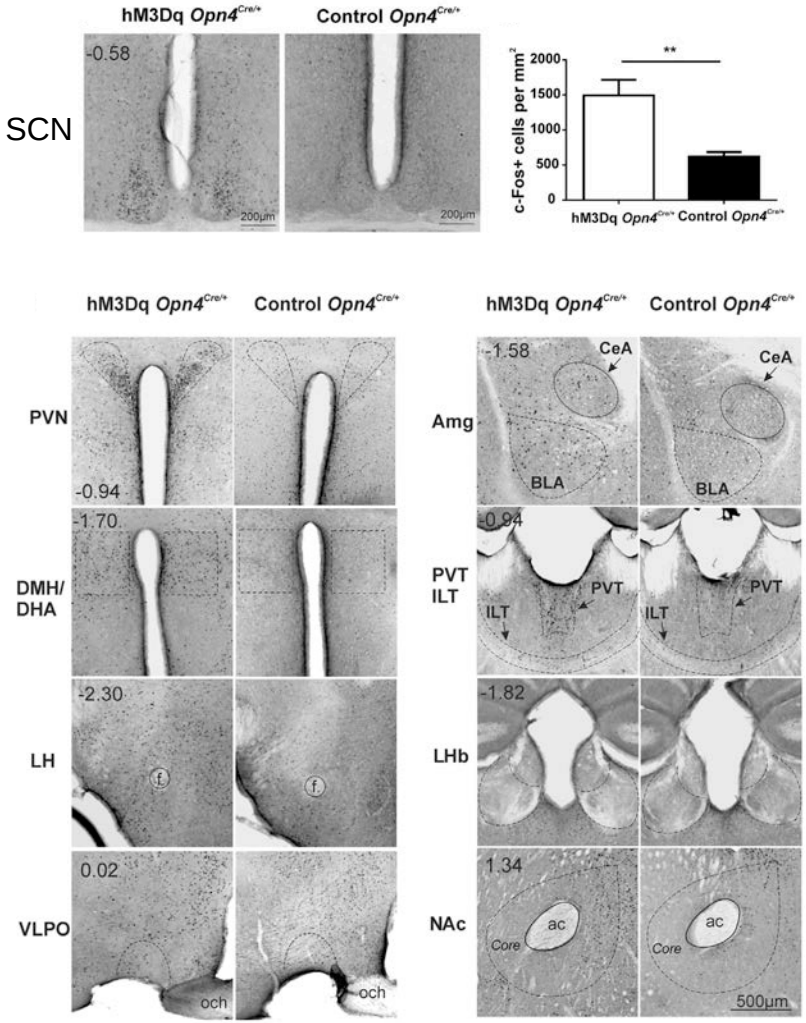
Chemogenetic activation of ipRGCs - Retina



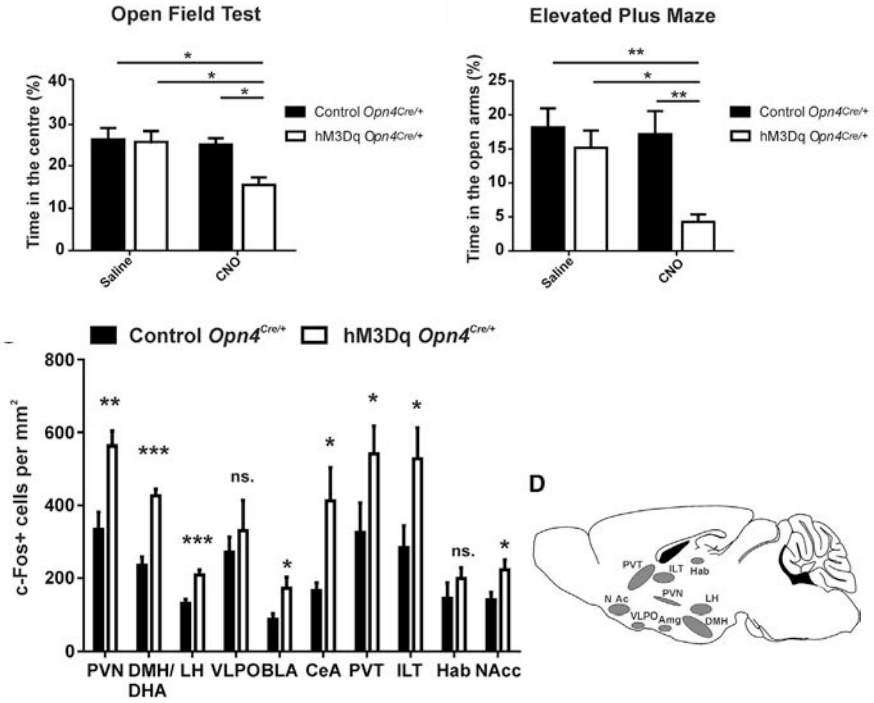
Using the paired flash technique, both rod and cone responses are suppressed by ipRGC activity induced by CNO injections. There is also evidence of reduced amacrine cell activity, with suppression of oscillatory potentials (not shown).

Milosavljevic et al., 2016, IOVS, 57(14):6305-12

Chemogenetic activation of ipRGCs - Brain



Chemogenetic activation of the hM3Dq receptor by CNO in the dark induces neural activity (as measured by c-Fos staining) in the SCN of hypothalamus and other brain regions associated with arousal / anxiety. This is also indicated by behavioural data from open field and elevated plus maze tests.

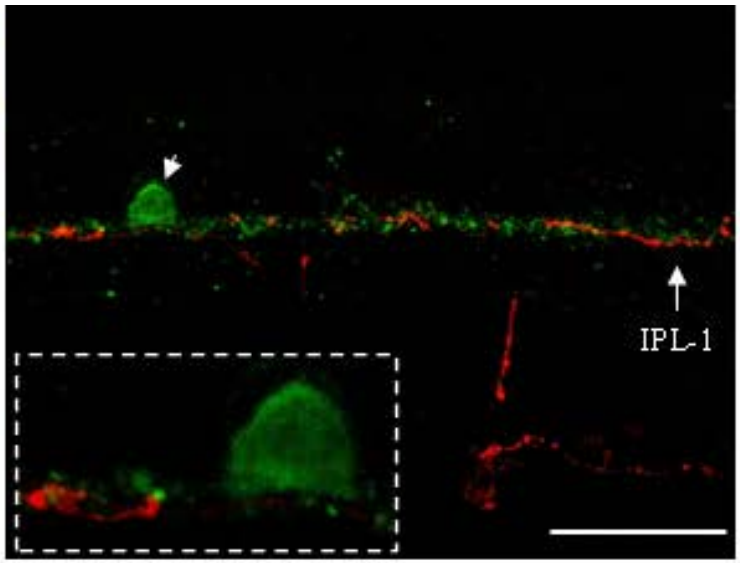


Milosavljevic et al., 2016, Curr. Biol, *26*: 2358-2363

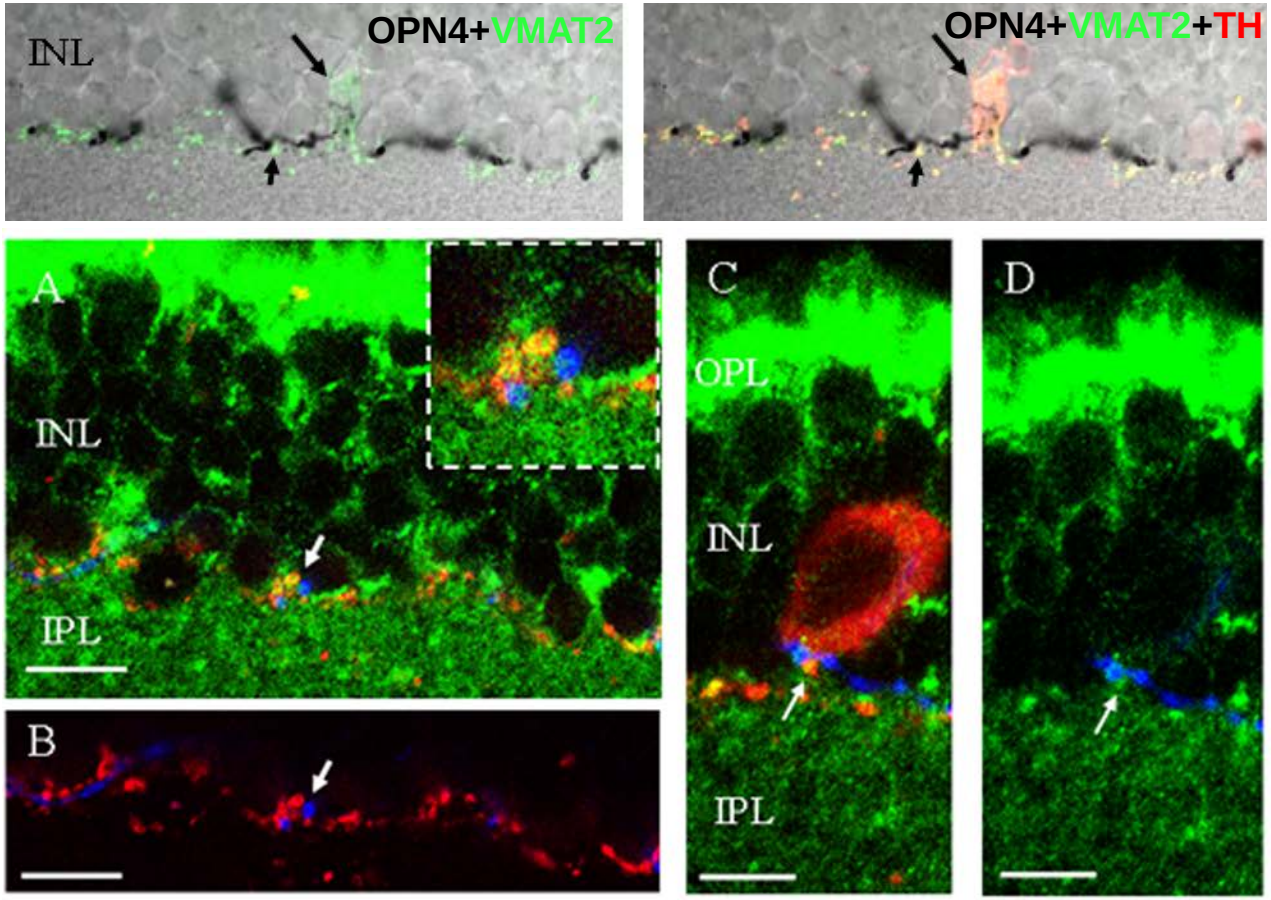
How could melanopsin influence the surrounding retinal circuits?

- ipRGCs signal to other retinal neurons via gap junctions
  - (Sekaran et al., Current Biol. (2003) 13, 1290-1298)
  - ipRGCs are electrically coupled to GABAergic amacrine cells in the RGC layer (Muller et al., J. Comp. Neurol. (2010) 518, 4813-4824)

- ipRGCs contact retinal dopamine neurons (Vugler, 2005)
  - Type of inter-plexiform neuron
  - In the retina, dopamine acts to light-adapt retinal circuitry and enhance visual acuity / contrast detection.
  - Retinal dopamine neurons traditionally thought to be driven by cone input…



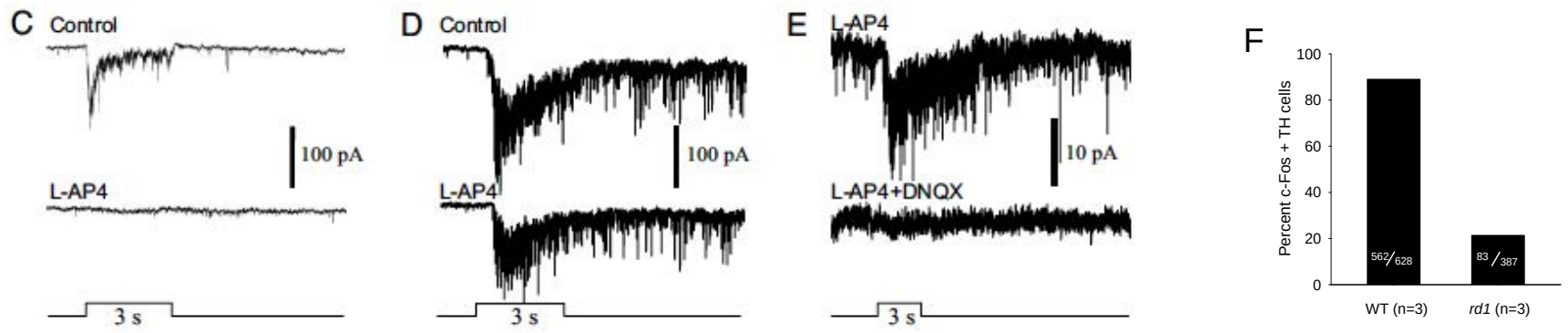
Dopamine neurons appeared largely presynaptic to ipRGCs



A-D: Tyrosine hydroxylase (red), melanopsin (blue), SNAP25 (green)

Vugler et al., Exp. Neurol. 2007, 205: 26-35

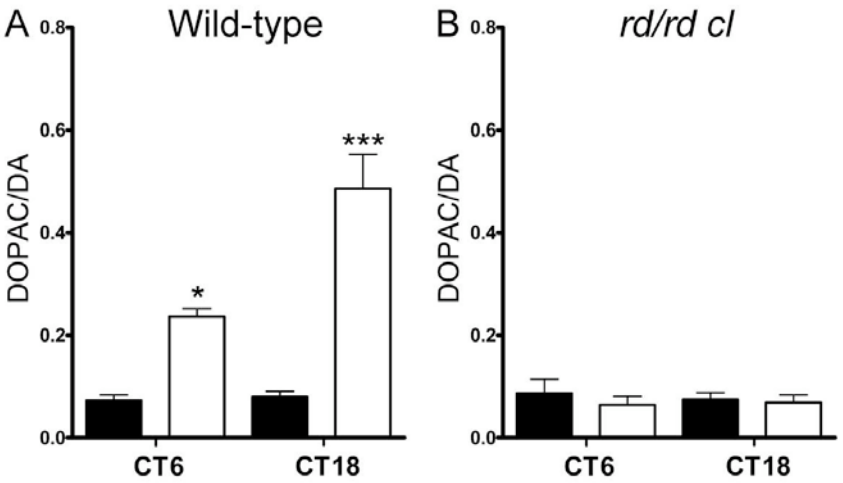
Electrophysiological evidence suggests “retrograde” intra-retinal signaling to dopamine neurons



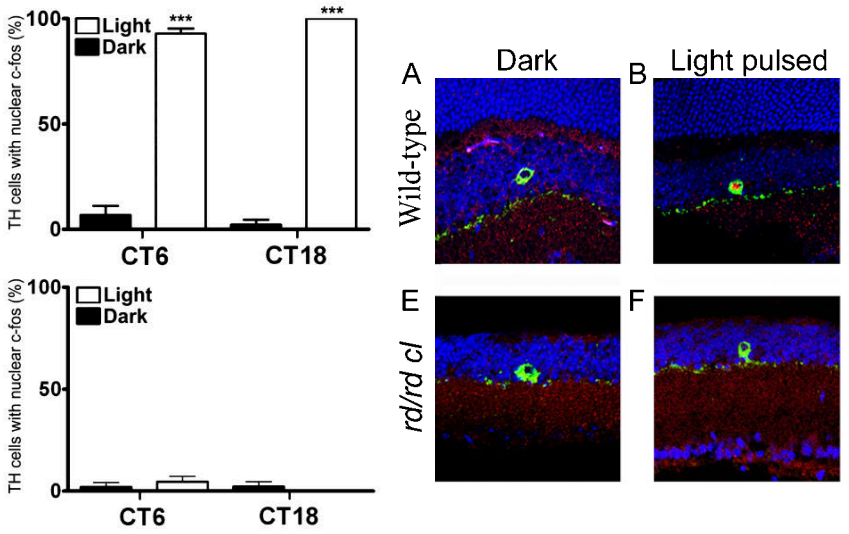
Two types of electrophysiological response found in dopamine neurons: transient (C) and sustained (D and E). The transient response elicited by a 3 second bright light stimulus can be abolished by application of 75µM L-AP4 (blocks signals from rods/cones). The sustained response is resistant to L-AP4 but abolished by co-application of 40µM DNQX (E). This implies that signaling from ipRGCs to dopamine neurons in the inner retina occurs and is mediated by AMPA/kainate-type glutamate receptors. They also reported that ~20% of dopamine (TH) cells are activated by light in both wildtype mice treated with L-AP4 and *rd* mice lacking rods (F).

(Zhang et al., (2008) *Proc. Natl. Acad. Sci. USA* 105(37) 14181-14186)

However, light stimulation fails to elevate dopamine turnover in mice lacking rods & cones



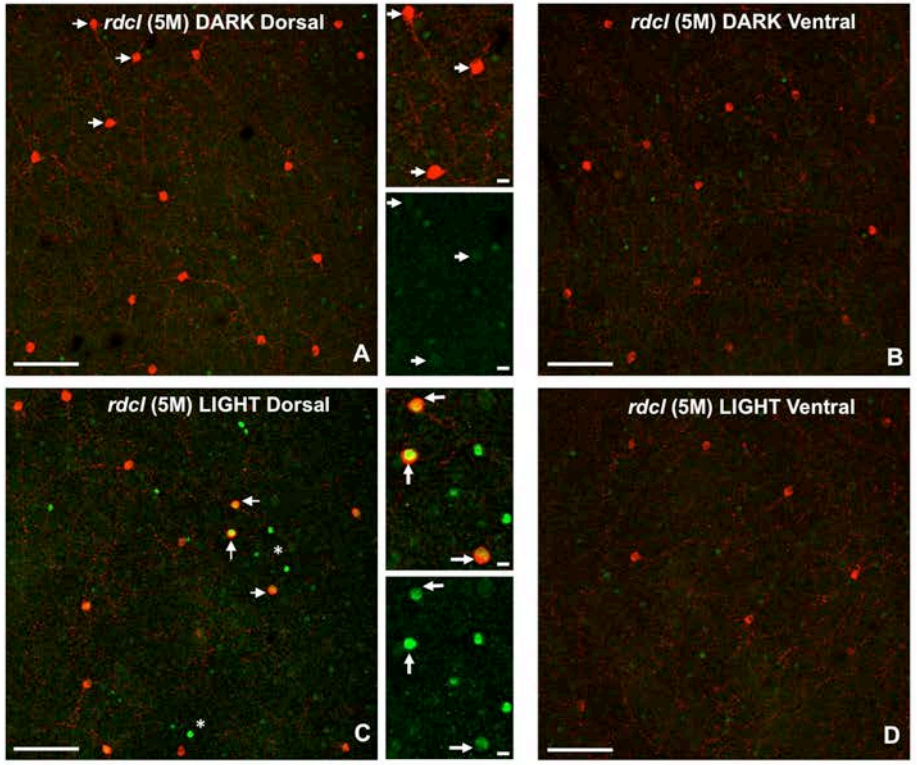
HPLC analysis showed no significant effect of light on dopamine turnover in mice lacking rods and cones (*rd/rd cl*).

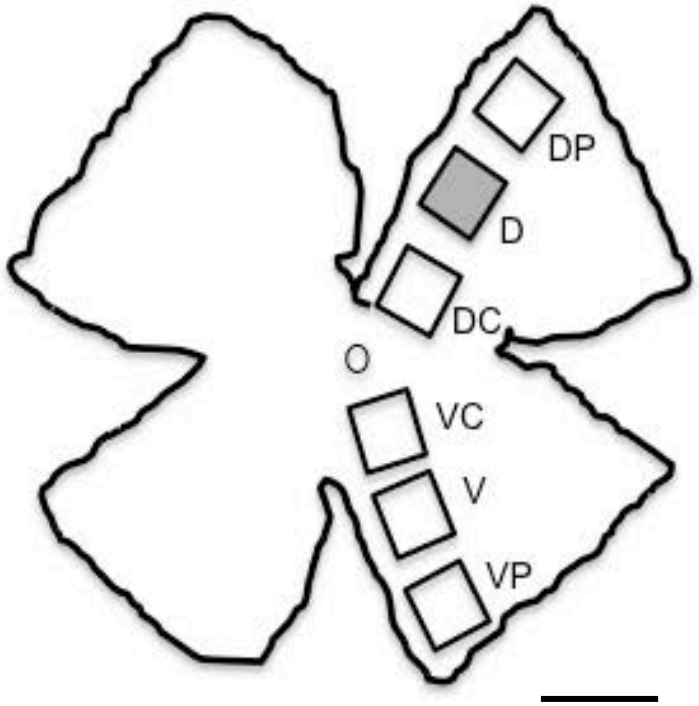


Functional anatomy using light-driven c-fos (red) in dopamine cells (green) showed no activation in mice lacking rods and cones (*rd/rd cl*).

Cameron et al., 2009, EJN, *29*: 761-767

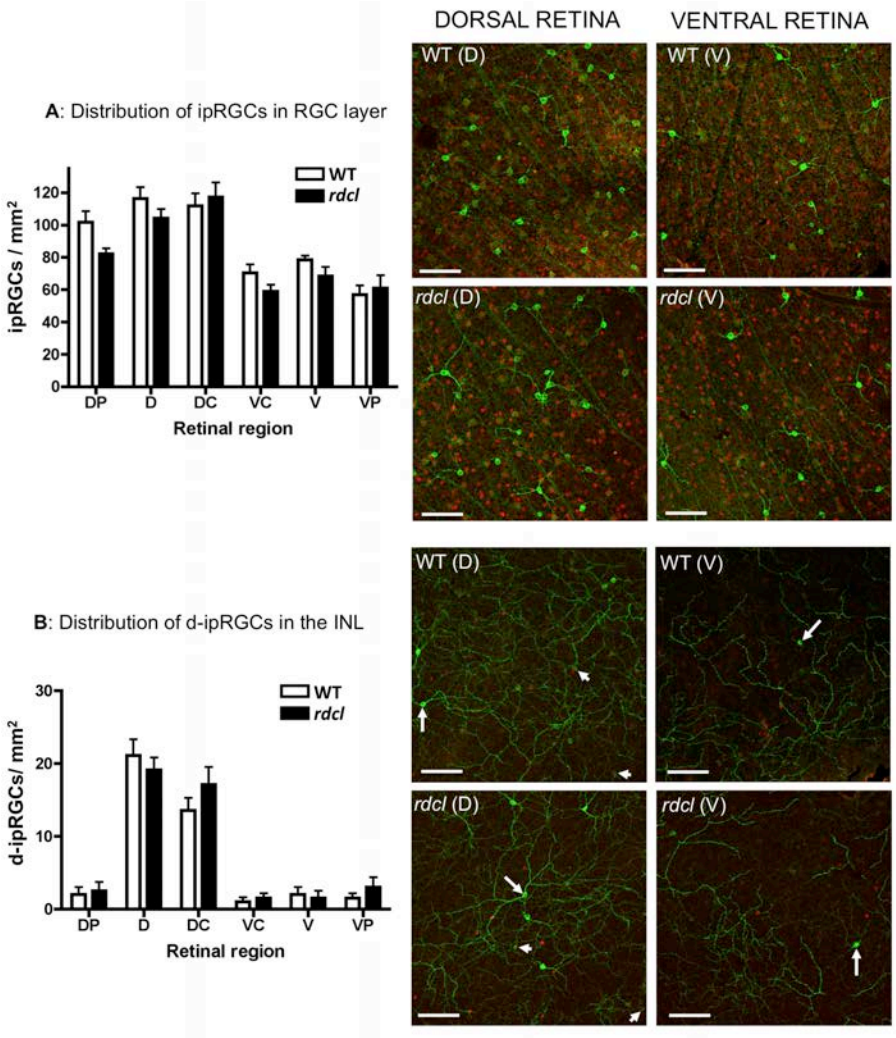
Retrograde intra-retinal signaling in mice lacking rods and cones occurs in a discrete region of dorsal retina

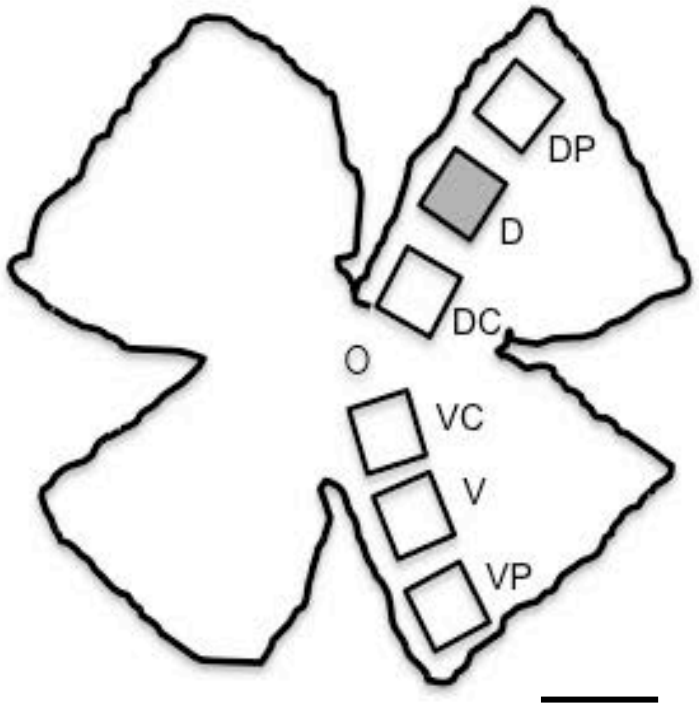




Semo et al., IOVS (2016) 57(1): 115-125

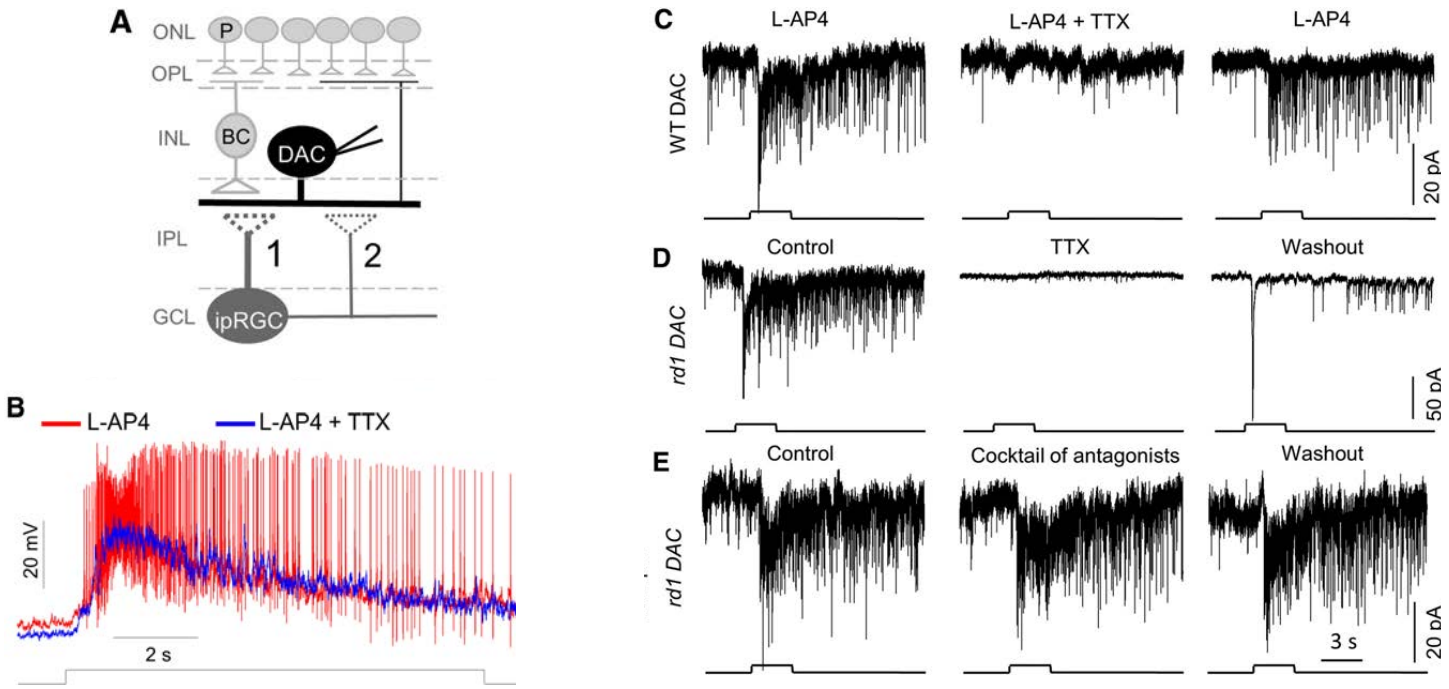
Retrograde intra-retinal signaling appears strongest where ipRGC density is highest





Semo et al., IOVS (2016) 57(1): 115-125

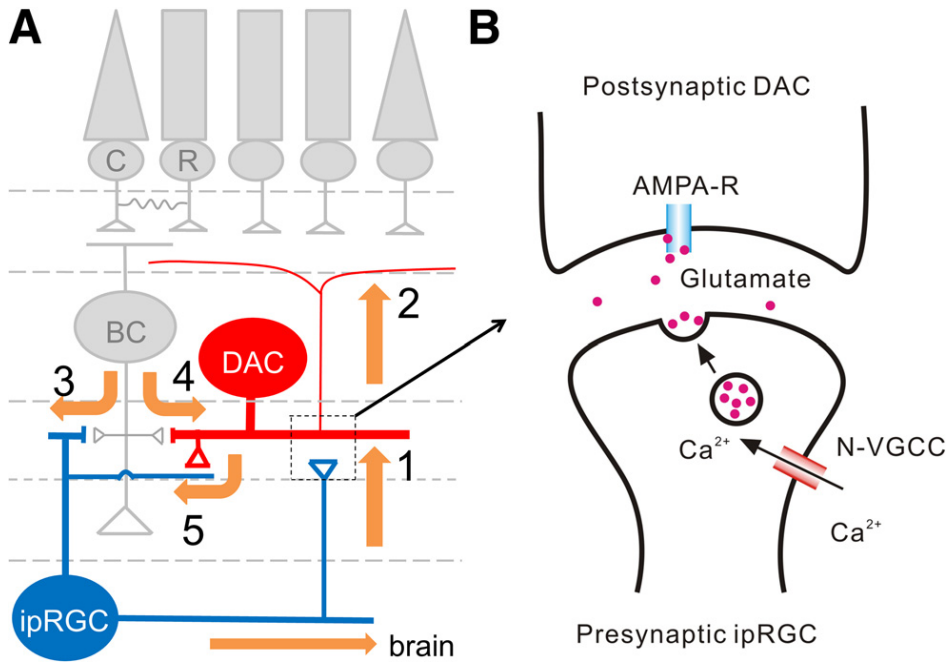
Intra-retinal retrograde signaling via ipRGC axon collaterals



Evidence for action potential transmission from ipRGCs to dopaminergic amacrine cells (DACs) **A**. Potential route of transmission: dendrodenritic (1) or axon collateral mediated (2). B. TTX removes action potentials from the ipRGC response but does not inhibit the light-Induced graded potential of ipRGCs. **C-D**, The intracellular sodium channel blocker QX314 was included in the pipette solution when recording from DACs to ensure that the voltage gated Na+ channels were already blocked within DACs prior to extracellular TTX application. **E**. Cocktail of GABA/Glycine receptor antagonists added In **E** to discount potential polysynaptic signaling from ipRGCs, via wide-field amacrine cells.

Prigge et al., 2016, J. Neurosci. 36(27): 7184-7197

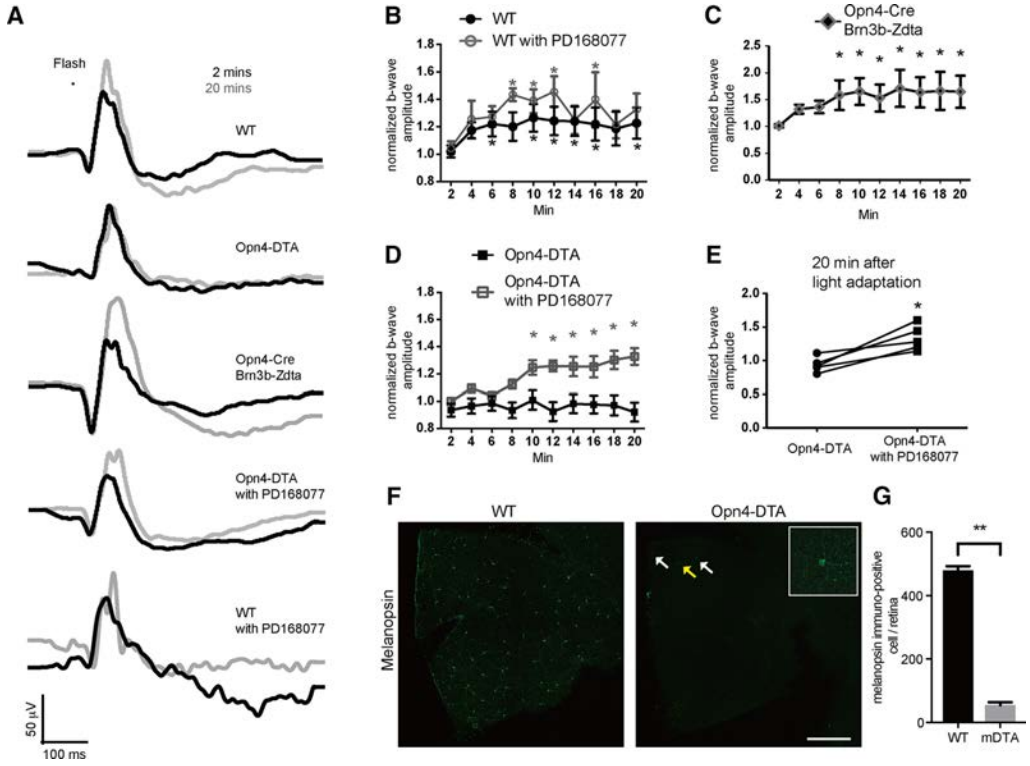
Intra-retinal retrograde signaling via ipRGC axon collaterals



Proposed neural pathways and synaptic mechanism underlying the ipRGC influence on light adaptation. **A**: Route 1, ipRGCs signal via axon collaterals to the dopaminergic amacrine cells (DAC), which then signal to outer retina via interplexiform axons. Routes 3 and 4 are proposed feedback pathways from rods / cones. Route 5 represents negative feedback from DACs back onto ipRGCs, whereby rod/cone signals could suppress ipRGC activity. **B** The proposed signaling mechanism involves N-type voltage gated calcium channels (N-VGCC) and AMPA type glutamate receptors.

Prigge et al., 2016, J. Neurosci. 36(27): 7184-7197

Dopamine mediates the effect of ipRGCs on the light-adapted ERG

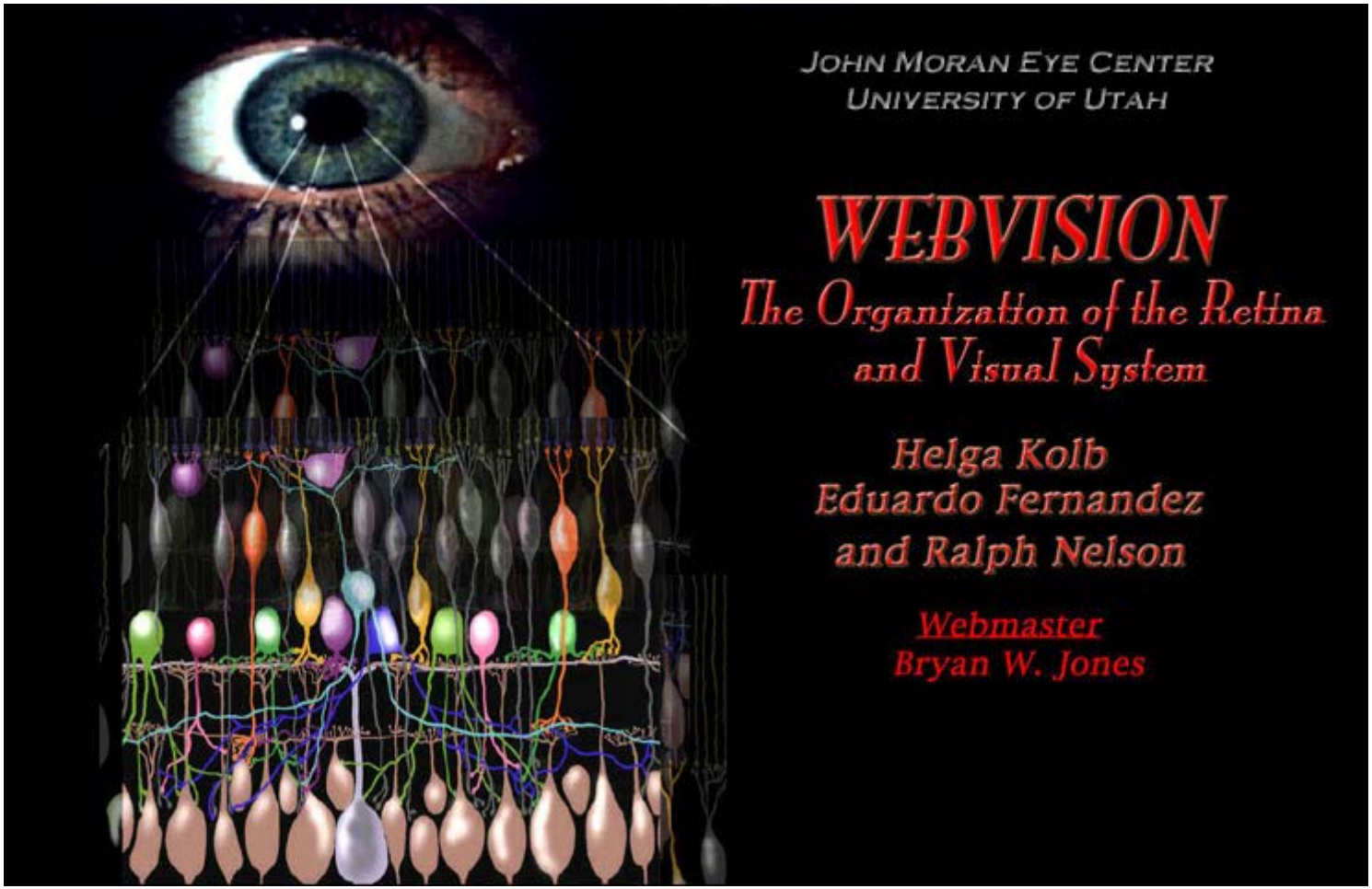


M1 ipRGCs modulate the light-adapted ERG b-wave via dopamine D4 receptors. Opn4-DTA mice have genetic ablation of ipRGCs, while Opn4-Cre Brn3b Zdta mice retain Brn3b negative ipRGCs (M1 ipRGCs). PD168077 is an agonist of D4 receptors.

Prigge et al., 2016, J. Neurosci. 36(27): 7184-7197

Summary:

- ipRGCs signal irradiance information to the brain
  - Integrating rod/cone signals with their own intrinsic light response
  - Their intrinsic light response is driven by melanopsin (Opn4)
- ipRGCs are a heterogeneous population of cells
  - In terms of morphology, physiology and connectivity to the brain.
- Melanopsin may support image-forming vision during daylight hours
  - By acting like a photographer's light meter, providing an independent measure of irradiance to light-adapt visual circuits.
  - Enhancing feature selectivity in both spatial & temporal dimensions.
  - This appears to occur at least in part in the retina (dopamine).
- **Melanopsin is involved in visual system development**
  - **This should be considered when interpreting data from studies relying upon *Opn4*$^{-/-}$ mice alone.**



http://webvision.med.utah.edu/